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Original Article

Internal Medicine Section

Clinical and Microbiological Profile of Tracheal Aspirates in Chronic Kidney Disease Patients

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ABSTRACT

Introduction: Chronic Kidney Disease (CKD) is a chronic inflammatory state, which inturn suppresses the immune system. CKD patients are hence at more risk for nosocomial infections. Ventilator Associated Pneumonia (VAP) is one of the major nosocomial infections. If not treated early and appropriately, it increases hospital stay and expenses in turn increasing morbidity and mortality. In recent times, due to irrational and rampant use of antibiotics, there is an emergence of multidrug resistant strains of organisms which are difficult to treat.

Aim: To study the clinical and microbiological profile of tracheal aspirate samples in CKD patients.

Materials and Methods: It was a retrospective cross sectional study conducted in the renal Intensive Care Unit (ICU) of a Tertiary Care Centre, Institute of Nephrourology, Bengaluru over a period of two years from July 2018 to July 2020. Clinical and demographic data of patients who fulfilled the inclusion criteria was recorded. Tracheal aspirates were obtained by convenient sampling technique. The samples were cultured on Blood Agar (BA), chocolate agar, MacConkey agar and Sabouraud's Dextrose Agar (SDA). Antibiotic sensitivity profiling was done by using Mueller Hinton agar. Statistical analysis was done using Statistical Package For The Social Sciences (SPSS) V23.0 software.

Results: Sixty-nine samples were analysed over a period of two years. Males (66.66%) were in majority. Diabetes Mellitus (64%) was the most common associated risk factor. Among the total samples obtained, around 70% showed bacterial growth. Gram negative bacteria (92.4%) were the most commonly isolated microorganism. Among the Gram-negative bacteria, *Acinetobacter baumanni* (45.3%) was the most common followed by *Klebsiella pneumoniae*. Majority of the gram negative organisms were sensitive to Polymyxin B, colistin, tigecycline. *Acinetobacter* and Klebsiella showed resistance to 3rd generation cephalosporins, aminoglycosides and fluoroquinolones.

Conclusion: There is emergence of extremely drug resistant gram negative organisms as the cause for VAP. They are dangerous and difficult to treat. They thus increase the hospital expenses by prolonging the hospital stay. Hence, the need of the hour is to formulate an appropriate antibiotic policy based on the population being treated and to follow strict infection control practices in ICU setup.

Keywords: Nosocomial infection, Renal disease, Ventilator associated pneumonia

INTRODUCTION

Chronic Kidney Disease (CKD) is a major public health problem to handle in developing nations like India [1]. Patients with CKD usually have other co-morbidities such as diabetes mellitus, hypertension which adds to both financial burden as well as increase in morbidity [1]. Complications of CKD are reduced immunity, anemia, malnutrition, inflammation, vitamin deficiencies and poor quality of life [2]. Also, patients on prolonged haemodialysis have reduced immunity and are more prone for nosocomial infections [3]. Nosocomial infection has been a burden on the healthcare system. It prolongs the duration of stay in the hospital. It increases the morbidity and mortality rate [4]. Ventilation associated pneumonia is one of the major types of nosocomial infections. It is associated with Endotracheal (ET) intubation and mechanical ventilation [5-8]. The tube present in the trachea causes ciliary damage and hence reduces bacterial clearance. Eventually, there is leakage around the tracheal cuff [9]. Reduced salivary flow and increased risk of mucositis in intubated patients also increased the risk for VAP [10]. There is also formation of biofilm around the ET tube. This predisposes to infections by Gram negative bacteria such as *Escherichia coli (E.coli)* and *Pseudomonas aeruginosa* [10,11]. The overall prevalence of VAP is between 4% to 28% [12]. Compared to patients who are not on ventilator, the incidence of pneumonia is 21 times higher in patients who are on ventilator [12-16].

The causative organisms vary from one place to another. Most commonly associated organisms are gram negative bacilli such as *Pseudomonas aeruginosa, Escherichia coli* (*E.coli*), *Acinetobacter baumannii, Klebsiella pneumoniae* [17] and gram-positive bacteria such as *Staphylococcus aureus* [18]. Due to inadvertent use of antibiotics, there has been a surge in multidrug resistant organisms such as carbapenem resistant bacteria in recent times [17]. Endotracheal Aspiration (ETA) helps in detecting the bacteria which is responsible for febrile episodes in mechanically ventilated patients and also VAP. It is easy to perform, reliable, inexpensive, minimally invasive and a bedside test. Healthcare workers can be easily trained to collect ETA [19]. There is no data on microbiological profile of tracheal aspirates in CKD population with VAP. Hence, the aim was to study the microbiological profile and antibiotic sensitivity pattern of tracheal aspirate cultures in mechanically ventilated CKD patients.

MATERIALS AND METHODS

This was a retrospective study conducted at Institute of Nephro-Urology, a tertiary care referral hospital inside Victoria Hospital Campus, Bangalore. The data from July 2018 to July 2020 was retrieved from patients' medical records. The process of data retrieving and analysis was performed from January 2020 to July 2020.

Inclusion criteria: All CKD patients admitted in Renal Intensive care unit

- CKD stage 3 and above
- On mechanical ventilation for a minimum of two calender days on the day of event, with day of ventilator placement being day one,
- Having a temperature of >38 degree Celsius or <36 degree Celsius.

- Leukopenia or leukocytosis
- Purulent tracheal secretions
- New onset radiographic features [13].

Exclusion criteria

- Patients who expired within of two calendar days on the day of event, with day of ventilator placement being Day one,
- Patients who were discharged against medical advice within 48 hours of mechanical ventilation
- Patients presenting with pneumonia at the time of admission.

A total of 69 samples of ETA were received in Microbiology laboratory and processed immediately using standard microbiological procedures ike microscopy, culture and antimicrobial susceptibility testing.

The samples were first subjected to Gram's staining and observed for the presence of number of pus cells and epithelial cells for assessing the clinical significance of sample as per Bartlett criteria, based on the relative number of Squamous Epithelial Cells (SEC) and inflammatory cells, seen microscopically per Low-power field (LPF). As per the criteria, a Q score which is a sum of +/- assigned values was calculated. A score of +2 is assigned, if White Blood Cells (WBCs) were more than 25/LPF and a score of +1, if 10-25 per LPF.

A score of -2 was assigned if the SEC were more than 25 per LPF and a score of -1 if 10 to 25 per LPF. A "+" Q score indicates that the quality of the specimen is acceptable, whereas a "-" or 0 Q score indicates excessive oral contamination and an unacceptable sample [20,21]. Irrespective of the Q score, the samples were processed using standard semi quantitative culture methods [22].

ETA samples were mechanically liquefied and homogenised by vortexing for one minute with 1 mL of 0.9% saline solution. The samples were then inoculated using semiquantitative calibrated loop method on 5% defibrinated sheep BA, Chocolate Agar (CA), MacConkey agar and SDA for quantifying the colony forming units per milliliter (cfu/mL) [22].

BA and chocolate agar plates were incubated in candle jar and the other plates in standard bacteriological incubator at 37°C. SDA plates were inoculated in duplicate and incubated at both, 37°C and at room temperature for up to one week as per standard guidelines [21,22]. Plates were evaluated for growth at 24 and 48 hours [22].

Following incubation, the colonies were quantified and identified using standard biochemical tests. Colony forming units of $\geq 10^5$ cfu/mL were considered significant for tracheal aspirates and $\geq 10^4$ cfu/mL for bronchoalveolar lavage [23].

All microorganisms were identified using standard laboratory methods [21]. Antimicrobial susceptibility testing was performed and interpreted by Kirby Bauer disc diffusion method as per current CLSI guidelines with appropriate quality control protocol [24].

STATISTICAL ANALYSIS

SPSS version 23.0 (IBM, Armonk, US) software was used for data entry and analysis. Frequencies and percentages were calculated using descriptive statistics. Tables and charts were constructed using Microsoft Excel and Word. Chi square test was used to calculate p-value for age and gender statistics. p-value less than 0.05 was considered as statistically significant.

RESULTS

A total of 69 patients with underlying CKD were included during the study period. Out of which, 46 (66.66%) were males and 23 (33.34%) were females [Table/Fig-1]. There was no significant difference between males and females among patients showing blood culture growths (p-value=0.793). Most of the patients were in the age group between 18 to 35 years (39.13%) [Table/Fig-2]. There was no significant difference based on age group (p-value=0.123).

Growth	Males	Females	Total
Present	27	15	42
Absent	19	8	27
Total	46	23	69

[Table/Fig-1]: Gender distrubution of study population.
Chi square test p-value=0.793

Age (Years)	Growth present	Growth absent	Total
18 to 35	16	11	27
36 to 50	15	2	17
More than 50	17	8	25
Total	48	21	69

[Table/Fig-2]: Age distrubution of study population.
Chi square test p-value=0.123

Predisposing risk factors such as Diabetes mellitus (64%) was seen in the majority of patients in present study, followed by alcohol abuse (45%) and hypertension (42%) [Table/Fig-3].

Risk factor	n (%)
Diabetes mellitus	44 (64)
Hypertension	29 (42)
COPD	18 (26)
Alcohol intake	31 (45)
Prolonged ICU stay	8 (12)

[Table/Fig-3]: Associated risk factors (N=69).
COPD: Chronic obstructive pulmonary disease

Out of the total 69 samples, 69.56% (48) were culture positive with significant bacterial growth (colony count $>10^5$/mL). Out of the positive cultures, 5 (10.41%) were polymicrobial and 43 (89.58%) were monomicrobial growths [Table/Fig-4].

Total growth n (%)	No growth n (%)
48 (69.56)	21 (30.43)

[Table/Fig-4]: Total number of tracheal culture.

The total isolates were 53. Predominant isolates were gram negative bacteria 49 (92.4%) followed by fungal species (6%) and gram-positive cocci (2%) [Table/Fig-5]. Among the gram negative bacteria, most common was *Acinetobacter baumannii* (45%), followed by *Klebsiella pneumoniae* (23%) and *Escherichia coli* (11%). Other less commonly found organisms were gram negative non fermenting bacilli (7%) and *Enterobacter species* (6%) [Table/Fig-5].

Organisms	n (%)
Acinetobacter species	24 (45)
Klebsiella pneumoniae	12 (23)
Escherichia coli	6 (11)
Non-fermenting gram negative bacilli	4 (7)
Enterobacter species	3 (6)
Staphylococcus aureus	1 (2)
Candida albicans	1 (2)
Candida non-albicans	1 (2)
Trichosporon species	1 (2)

[Table/Fig-5]: Microbiological profile of tracheal aspirates (N=53).

Most of the gram-negative isolates were multidrug resistant. A total of 77.55% of the isolates were resistant to amikacin, 91.84% were resistant to cefotaxime and ceftazidime (3rd generation cephalosporins), 91.84% were resistant to amoxicillin-clavulinic acid (betalactam + betalactamase inhibitor combination) and 89.8% were resistant to ciprofloxacin (fluoroquinolone). Meropenem (carbapenem) resistance accounted to 73.5% [Table/Fig-6].

Antibiotics	Sensitive n (%)	Resistant n (%)
Amikacin	11 (22.45)	38 (77.55)
Gentamycin	7 (14.29)	42 (85.71)
Amoxicillin-Clavulinic acid	4 (8.16)	45 (91.84)
Cefotaxime (3rd generation Cephalosporins)	4 (8.16)	45 (91.84)
Ceftazidime (3rd Generation Cephalosporins)	4 (8.16)	45 (91.84)
Aztreonam	4 (8.16)	45 (91.84)
Ciprofloxacin	5 (10.20)	44 (89.80)
Levofloxacin	10 (22.40)	39 (77.60)
Cefoperazone-Sulbactum	20 (40.80)	29 (59.20)
Piperacillin-Tazobactum	17 (34.70)	32 (65.30)
Ertapenem	16 (32.65)	33 (67.35)
Imipenem	12 (24.50)	37 (75.50)
Meropenem	13 (26.50)	36 (73.50)
Colistin	49 (100)	0 (0.00)
Polymyxin B	49 (100)	0 (0.00)
Tigecycline	48 (97.95)	1 (2.04)

[Table/Fig-6]: Antibiogram of gram-negative isolates (N=49).

Among the 24 isolates of *Acinetobacter baumanii*, 95.8% was resistant to amikacin and gentamycin, cefotaxime, ceftazidime, amoxicillin-clavulinic acid and ciprofloxacin. Resistance to meropenem and imipenem (carbapenems) was seen in 83.68% of isolates [Table/Fig-7]. Among the 12 isolates of *Klebsiella pneumoniae*, all were resistant to amikacin, gentamycin (aminoglycosides), cefotaxime, ceftazidime, amoxicillin-clavulanic acid and ciprofloxacin. Resistance to meropenem, imipenem, ertapenem was 100% [Table/Fig-7]. *E.coli* showed variable sensitivity and resistance pattern. 16.6% resistant to amikacin and meropenem, 100% resistant to cefotaxime, ceftazidime and ciprofloxacin. [Table/Fig-7]. All isolates were sensitive to polymyxin B and colistin [Table/Fig-7].

Antibiotics	*Acinetobacter species* n (%)	*Klebsiella pneumoniae* n (%)	*Escherichia coli* n (%)	Non-fermenting gram negative bacilli n (%)	*Enterobacter species* n (%)
Amikacin (Aminoglycoside)	1 (4.20)	0 (0)	5 (83.33)	2 (50)	3 (100)
Gentamycin (Aminoglycoside)	1 (4.20)	0 (0)	3 (50)	1 (25)	2 (66.67)
Amoxycillin-Clavulinic Acid (Betalactam + Betalactamase Inhibitor Combination)	NA	0 (0)	1 (16.66)	NA	3 (100)
3rd Generation Cephalosporin (Cefotaxime)	1 (4.20)	0 (0)	0 (0)	0 (0)	3 (100)
3rd Generation Cephalosporin (Ceftazidime)	1 (4.20)	0 (0)	0 (0)	0 (0)	3 (100)
Aztreonam (Monobactam)	1 (4.20)	0 (0)	0 (0)	0 (0)	3 (100)
Ciprofloxacin (Fluoroquinolone)	1 (4.20)	0 (0)	0 (0)	1 (25)	3 (100)
Levofloxacin (Fluoroquinolone)	4 (16.80)	0 (0)	1 (16.66)	2 (50)	3 (100)
Cefoperazone-Sulbactum (Third-Generation Cephalosporin and Beta Lactamase Inhibitor)	9 (37.50)	0 (0)	5 (83.33)	3 (75)	3 (100)
Piperacillin-Tazobactum (Penicillin and Beta Lactamase Inhibitor)	10 (41.66)	0 (0)	3 (50)	1 (25)	3 (100)
Ertapenem (Carbapenem)	8 (33.33)	0 (0)	3 (50)	2 (50)	3 (100)
Imipenem (Carbapenem)	4 (16.32)	0 (0)	4 (66.67)	1 (25)	3 (100)
Meropenem (Carbapenem)	4 (16.32)	0 (0)	5 (83.33)	1 (25)	3 (100)
Colistin (Colistimethate)	24 (100)	12 (100)	6 (100)	4 (100)	3 (100)
Polymyxin B	24 (100)	12 (100)	6 (100)	4 (100)	3 (100)
Tigecycline (Glycylcycline)	23 (95.83)	12 (100)	6 (100)	4 (100)	3 (100)

[Table/Fig-7]: Antibiotic sensitivity pattern of gram-negative bacteria.

All isolates of *E.coli*, *Klebsiella pneumonia* and *Enterobacter species* were sensitive to tigecycline. However, the study found one isolate of *Acinetobacter baumannii* which was resistant to tigecycline (4.17%) [Table/Fig-7]. Among the gram positive bacteria, only one strain of methicillin sensitive *Staphylococcus aureus* was isolated.

Among the fungal isolates, one isolate each of *Candida albicans*, *Candida non-albicans* and *Trichosporon species* were isolated. A 33.33% of *Candida species* showed resistance to fluconazole. All were sensitive to voriconazole, amphotericin B, caspofungin and micafungin [Table/Fig-8].

Antifungals	Sensitive n (%)	Resistant n (%)
Fluconazole	2 (66.70)	1 (33.30)
Voriconazole	3 (100)	0
Amphotericin B	3 (100)	0
Caspofungin	3 (100)	0
Micafungin	3 (100)	0

[Table/Fig-8]: Antifungal sensitivity of fungal isolates.

Blood culture of ETA samples which did not isolate any organism was performed. No organisms were isolated. Five bacteria were isolated from blood samples of patients whose ETA samples isolated predominantly single bacteria [Table/Fig-9]. Only two of these blood cultures grew the same bacteria as the ETA culture (*Acinetobacter baumanii* and *E.coli*) [Table/Fig-10].

ETA culture result		Number of positive blood culture/Total number of culture n (%)
No growth	21	0 (0)
Polymicrobial growth	5	1 (0.14)
Predominant growth of only one pathogen	43	5 (7.24)

[Table/Fig-9]: Entotracheal Aspirate (ETA) culture and blood culture results.

Growth on ETA culture	Growth on blood culture
Klebsiella Pneumoniae	*E.coli*
Klebsiella Pneumoniae	*Candida non-albicans*
Acinetobacter baumanii	*Acinetobacter baumanii*
Non-fermenting gram negative Bacilli	*Candida non-albicans*
E.coli	*E.coli*

[Table/Fig-10]: Positive blood culture whose ETA grew predominant pathogen.
ETA: Entotracheal aspirate

DISCUSSION

Offlate, resistant strains are emerging in the clinical setting due to inadvertent use of antibiotics [25]. These resistant strains are a predominant cause of nosocomial infection of patients admitted in intensive care unit. Patients in ICU are subjected to invasive procedures such as mechanical ventilation which increases the risk

of nosocomial infections [26]. If not treated early, VAP is associated with severe morbidity and mortality. For early and appropriate treatment, it is important that we have a good empirical antibiotic policy. Aetiological agents vary widely based on the study population. Predisposing risk factors such as Diabetes Mellitus (DM) (64%) [Table/Fig-3] was seen in the majority of patients in present study. In a study, by Dey A and Bairy I, [17], a total of 28 patients had co-morbidities out of which 15 (60%) had VAP. In another study by Rajasekhar T et al., prolonged hospital stay (73%) was the most common risk factor [27]. DM is the most common cause of CKD and is a proinflammatory state. Hence, it is the most common risk factor in this study. This study had a positive culture rate of 69.56% [Table/Fig-4]. A study by Simoni P et al., had a positive culture rate of 100% [28]. Cardenosa Cendrero JA et al., had a positive culture rate of 89% [29]. This difference in culture positivity rate can be due to the different study population or colonisation of the ET tube [30].

This study showed predominant growth of gram negative bacilli (92%) compared to gram positive cocci (2%) [Table/Fig-5]. This was in concordance with studies by Gupta P et al., and Chandra D et al., where in 86% and 85% of isolates were gram negative, respectively [31,32]. Also, a study by Ebenezer R et al., in burns patients demonstrated higher gram negative tracheal isolates compared to gram positive isolates [33]. There is increased incidence of gram negative species in nosocomial infections. They are difficult to treat and hence cause prolonged ICU stay. *Acinetobacter baumannii* was the predominant isolate in this study. In other studies by Caskurlu H et al., and Swati A et al., *Acinetobacter* species was the most common isolate [34,35]. This is due to its ability to spread easily by aerosols and high degree of resistance to the surrounding environment and antimicrobials [36].

The second most common isolate was *Klebsiella pneumoniae* followed by *E.coli* in this study. In a study, by Chandra D et al., Klebsiella (32.35%) was the second most common bacteria isolated [32]. In this study, all isolates of *Acinetobacter baumannii* were sensitive to Colistin and Polymyxin B (100%) [Table/Fig-7]. It showed 95.83% sensitivity to tigecycline and 37.5% sensitivity to cefoperazone-sulbactam. A study by Malik M et al., demonstrated 69% sensitivity to cefoperazone-sulbactam [37]. Anusha N et al., showed *Acinetobacter* was highly sensitive to ciprofloxacin and imipenem [38]. The emergence of multidrug resistant strains may be due to inadvertent use of antibiotics. This study showed *Klebsiella pneumoniae* to be 100% sensitive to colistin, polymyxin B and tigecycline [Table/Fig-7]. Ahmad H et al., showed *Klebsiella pneumoniae* to be sensitive to cefoperazone- sulbactam (81.8%) and piperacillin-tazobactam (71.4%) in their study [39]. Decrease in sensitivity of Klebsiella may be attributed to cross infections and unjust use of antibiotics [40]. In this study, other gram-negative bacilli such as *E.coli* and Enterobacter showed variable sensitivity profile. *E.coli* showed 83.33% sensitivity to aminoglycosides, cefaperazone-sulbactam and carbapenems. A study by Anusha N et al., showed similar results. *E.coli* was sensitive to amikacin and meropenem in their study [38].

In the total duration of two years, the study found only one isolate of gram-positive cocci, which was methicillin sensitive staphylococcus aureus. This is consistent with the study results of Gupta P et al., [31]. Analysis of the resistance profile in this study, showed *Acinetobacter* to be 95.8% resistant to aminoglycosides, third generation cephalosporins and fluoroquinolones. A 83.68% were resistant to carbapenems. These results were consistent with the study results of Gupta P et al., and Ahmad H et al., [31,39]. One isolate of *Acinetobacter* was resistant to tigecycline in present study. This denotes an emergence of an alarming situation, wherein there is increased incidence of multidrug resistant and extremely drug resistant microorganisms in the tracheal aspirates which in turn increases their morbidity and mortality [17]. Other gram-negative isolates such as *Klebsiella pneumoniae* showed 100% resistance to aminoglycosides, third generation cephalosporins, fluoroquinolones and carbapenems. *E.coli* and Enterobacter showed variable resistance patterns. They were sensitive to broad spectrum antibiotics. This is in concordance with the studies by Gupta P et al., and Malik M et al., [31,37]. This points to the fact that there is an increase in the rates of carbapenem resistance.

The number of islolates from blood cultures were different when compared to ETA samples which predominantly showed single isolate. However, same organism as that of the ETA cultures was isolated from only two blood culture [Table/Fig-9,10]. Other studies have demonstrated a correlation between 0% to 80% [41,42]. ICU infections are most commonly caused by multidrug resistant organisms. CKD population is already immunocompromised and increasingly susceptible for infections as they have associated comorbidities such as diabetes and are at increased risk of exposure to pathogens due to frequent hospital visits. The antibiogram of these isolates should be periodically monitored and the empirical treatment protocols updated regularly. Every center should study the antimicrobial resistance patterns prevalent in their ICU and enforce infection control practices.

Limitation(s)

This study was a single centre experience. Extrapolation of the results to the entire population may be questionable.

CONCLUSION(S)

It is clear from the previous and present studies that the resistance pattern of organisms causing VAP has been changing along with time. It varies between geographical areas and also between the study population. CKD population is at increased risk of infection. Off late, there has been an emergence of multidrug resistant strains due to which the treatment of VAP in CKD has become a major challenge. There is an increase in Extended spectrum beta-lactamase (ESBL), Metallo-beta-lactamase (MBL) and AmpC production by Gram-negative Bacteria (GNB) in CKD population. There has been a trend towards an increase in VAP due to preexisting comorbidities in CKD patients and prolonged intubation. Hence, the antibiotic policy needs regular updating for CKD patients. The need of the hour is to prevent VAP in CKD population caused by multidrug resistant strains by formulating appropriate empirical antibiotic policy and also hospital infection control practices.

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