



**Full Length Research Article**

**BACTERIAL COLONIZATION IN TRACHEAL TUBES OF INTENSIVE CARE UNIT PATIENTS: STUDY FROM A TERTIARY CARE CENTRE**

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**ABSTRACT**

**Introduction** – Invasive diagnostic and therapeutic methods have saved many lives. On the other hand it can cause some life threatening consequences due to infections. Bacterial infection in the lower respiratory tracts remains a main complication of tracheal intubation. The incidence of nosocomial pneumonia varies from 9% to 68%. Knowledge of susceptibility pattern is helpful in selecting the empirical therapy.

**Objectives** - To determine prevalence and antibiotic susceptibility profile of bacteria colonizing tracheal tubes in the ICU.

**Methodology** - The study was carried out for a period of 3 years during 2012- 2014. Data of total 5319 samples for culture and sensitivity were recorded in WHONET 5.6 and analyzed. Colony count of 10<sup>5</sup>cfu/mL is considered significant. Culture pairs were assessed for change in (1) species of bacteria isolated and (2) change in empiric antibiotic coverage. The results were analyzed using appropriate statistical methods.

**Results** - Culture of 5319 samples yielded 3268 isolates from 1232 patients. Positive culture rate was 55%. The most frequently isolated organism was *Acinetobacter baumannii* followed by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *E.coli*, *Proteus mirabilis*. High level of resistance to imipenem (ranged 30%-60%) and meropenem (ranged 50%-70%) is an alarming sign. Colistin resistant was not detected. *Staphylococcus aureus* was isolated with >60% MRSA rates. Change in flora was present in around 72 % patients. Additional antibiotic coverage was needed in around half of the patients.

**Conclusions** - This study presents the most common microorganisms colonized and their antibiotic resistance pattern. The variability of flora and the potential need to adjust antibiotic coverage based on culture data suggest that surveillance tracheal aspirates are important during exacerbations.

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**INTRODUCTION**

The intensive care unit (ICU) often is called the epicenter of infections due to its extremely vulnerable population of reduced host defenses deregulating the immune responses and increased risk of becoming infected through multiple procedures. The use of invasive devices namely intubation, mechanical ventilation, vascular access etc. distorts the anatomical integrity and protective barriers of patients. Administration of several drugs (sedatives, muscle relaxants) also predispose for infections by reducing the cough and

swallow reflexes or by distorting the normal non-pathogenic bacterial flora. Consequently, the ICU population has one of the highest occurrence rates of nosocomial infections (20-30% of all ICU admissions) leading to an enormous impact on morbidity, hospital costs, and often, survival (Mondal *et al.*, 2013). Hospital personnel and the environment can be the microbial source. During last two decades, use of invasive diagnostic and therapeutic methods has saved many lives. On the other hand it can cause some life threatening consequences due to severe persistence and resistance infections (Amini *et al.*, 2009). Patients with mechanical ventilation have an increased risk for respiratory tract infection because the tube which has been inserted into the trachea reduces the clearance of bacteria and increases the leakage of secretion around the

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cuff of the tube and disable the ciliary tract by damaging it (Abdollahi *et al.*, 2013). Bacterial infection due to gram-negative bacilli in the lower respiratory tracts remains a main complication of tracheal intubation in patients requiring ventilator equipments (Khosravi *et al.*, 2012). Various studies have proposed different causative microorganism as the most common etiology for intubation related respiratory infections including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and methicillin resistant *Staphylococcus aureus* (MRSA) and *S. aureus* (Abdollahi *et al.*, 2013).

In consequence, nosocomial pneumonia is a common and a life threatening problem among seriously ill patients who are mechanically ventilated. The incidence varies from 9% to 68% with a high fatality rate ranging from 50% to 80%, especially when it is caused by antibiotic-resistant bacteria (Khosravi *et al.*, 2012). Routine endotracheal aspirate cultures of critically ill patients in ICU may be predictive of patients who are at high risk of invasive disease, and may guide the selection of appropriate empirical therapy based on the predominant pathogen identified in the cultures in the development of VAP (Joseph *et al.*, 2010). However, in adult patients the cause of respiratory exacerbations is usually related to the acquisition of new strains of bacteria, rather than an increase in the number of bacteria present when clinically stable (Cline *et al.*, 2012). Failure to treat the potential pathogen increases the morbidity and mortality, while overenthusiastic treatment of colonizing organisms results in unnecessary exposure to broad spectrum antibiotics and predisposes to infection with multidrug resistant (MDR) pathogens (Joseph *et al.*, 2010). The objective of this study was to determine prevalence and antibiotic susceptibility profile of bacteria colonizing tracheal tubes to different antibiotics in the intensive care unit of a tertiary care center, New Delhi, India.

## MATERIAL AND METHODS

The study was carried out for a period of 3 years during 2012-2014. This ICU is 8 bedded open medical-surgical unit with two isolation rooms. Patients are admitted from within the hospital and directly from the community or from other hospitals through the accident and emergency department. The ICU has bed occupancy of 100%. Patient to nurse ratio is 3.3:1 in day time and 5:1 at night. Data of total 5319 tracheal samples for culture and sensitivity were recorded in WHONET 5.6 and analyzed. The pattern of microorganism and bacterial resistance to antibiotics was recorded.

### Sample Collection

Tracheal aspirates were routinely submitted on every third day at the department of microbiology for bacteriological examination from the adult patients of ICU. They were inoculated on 5% Sheep Blood Agar (SBA) and MacConkey agar media by standard loop as soon as they were received. Colony count of  $10^5$  cfu/mL is considered significant (Joseph *et al.*, 2010). Identification of bacteria was carried out by conventional biochemical tests (Winn *et al.*, 2006).

### Antimicrobial Susceptibility Test

Antimicrobial susceptibility pattern was performed using disk diffusion method on Muller Hinton agar plate. The isolates were tested against penicillin, ceftazidime, netilmicin,

imipenem, meropenem, ertapenem, ciprofloxacin, amikacin, tazobactam/piperacillin, cefoperazone/sulbactam, gentamicin, clindamycin, vancomycin, ceftazidime, and erythromycin. Results were interpreted as per CLSI guidelines 2014 (Clinical and Laboratory Standard Institute, 2014). Empirical antibiotic policy for respiratory tract infections in ICU and VAP in our hospital for possible pathogens *Acinetobacter* spp., *Pseudomonas* spp., *E. coli*, *Klebsiella* spp., *Enterobacter* spp. and *Staphylococcus aureus* includes Cefoperazone+Sulbactam OR Piperacillin+Tazobactam OR Imipenem OR Meropenem + Linezolid or Vancomycin.

### Culture pairs

We also received two or more than two samples from a single patient. A total of 501 Culture pairs were assessed for change in (1) species of microbes isolated (i.e., no changes, new species identified, loss of a previously isolated species, or both gain and loss of species in the second culture); and (2) change in empiric antibiotic coverage that would be needed to treat the microbes present in the second culture, compared with the first time culture. The results of the second culture were considered to require additional antibiotics when any of the following conditions were present: (1) presence of gram-positive bacteria regardless of susceptibility, when this species was not present in any of the culture; (2) change in *Staphylococcus aureus* from methicillin susceptible to resistant or from methicillin resistant, clindamycin susceptible to clindamycin resistant; or (3) presence of resistance in new or persisting gram-negative species that would not have been covered by appropriately empirical antibiotic policy for the susceptibilities of the gram-negative microbe(s) in the first culture.

If more than 2 samples were received from a single patient then earliest sample with change in flora is considered as second culture. The susceptibilities of each isolate were recorded. The interval between cultures was determined from the dates the cultures were collected.

### Statistical analysis

The results were analyzed using appropriate statistical methods. Data of prevalence and antibiotic susceptibility profile of bacteria were analyzed using WHONET 5.6.  $\chi^2$  or Fisher exact test methods were used for comparisons of categorical data. All *P* values were two tailed; *P* value <0.05 was considered statistically significant.

## RESULTS

Culture of 5319 samples of tracheal aspirate was done during 2012 to 2014. A total of 2936 (55%) sample showed significant growth. Frequency distribution of the organisms is shown in Table 1. A total of 3268 (1063 in 2012, 1317 in 2013, 886 in 2014) isolates were recovered from 1232 patients (392 in 2012, 533 in 2013 and 307 in 2014). Among them 3184 (1038 in 2012, 1275 in 2013, 871 in 2014) were gram-negative bacteria, 75 (22 in 2012, 38 in 2013, 15 in 2014) were gram-positive bacteria and 9 (3 in 2012, 4 in 2013, 2 in 2014) were *Candida* spp. Among gram-negative bacteria most frequently isolated organism was *Acinetobacter baumannii*

(49.5%, 49.1%, 51.5% in 2012, 2013, 2014 respectively) followed by *Pseudomonas aeruginosa* (26%, 14.7%, 21.9% in 2012, 2013, 2014 respectively), *Klebsiella pneumoniae* (14.7%, 11.5%, 17.1% in 2012, 2013, 2014 respectively), *E.coli* (4%, 4.5%, 2.7% in 2012, 2013, 2014 respectively), *Proteus mirabilis* (2.6%, 1.8%, 2.7% in 2012, 2013, 2014 respectively), *Klebsiella oxytoca* (0.3%, 1.1%, 1.6% in 2012, 2013, 2014 respectively) and *Citrobacter freundii* (0.3%, 0.5%, 0.7% in 2012, 2013, 2014 respectively). Among gram-positive bacteria most frequent organism was *Staphylococcus aureus* (1.8%, 2.6%, and 1.5% in 2012, 2013, and 2014 respectively) followed by *Enterococcus faecalis* (0.3%, 0.2%, and 0.2% in 2012, 2013, and 2014 respectively).

ceftazidime was significantly increased in 2014 (96%) in comparison to 2012 (79%) with no significant change in 2013. *E.coli* showed overall >75% resistance for ceftazidime, >35% resistance for piperacillin/tazobactam and >40% resistance for amikacin with no significant change. Resistance to imipenem was 13%, 8%, 6% in 2012, 2013, and 2014 respectively with no significant change. There was significant increase in meropenem resistance in 2013 (36%) in comparison to 2012 (14%) and 2014 (18%). Resistance to cefoperazone/sulbactam was significantly increased in 2014 (53%) in comparison to 2013 (28%) and 2012 (31%). There was significant increase in ciprofloxacin resistance in 2014 (94%) in comparison to 2012 (83%) with no significant change in 2013(89%).

**Table 1. Frequency Distribution of Bacterial Species along with Fungi Present in Tracheal Aspirates**

Organism	No. of isolates (%)				Total no. of isolates (n=3268 from 1232 patients)				
	2012	2013	2014	total	2012	2013	2014	Total	
Gram negative bacteria (n=3184)	<i>A.baumannii</i>	526 (49.5)	646 (49.1)	457 (51.5)	1629	1038	1275	871	3184
	<i>P. aeruginosa</i>	276 (26)	290 (14.7)	194 (21.9)	760				
	<i>K. pneumoniae</i>	157 (14.7)	248 (11.5)	152 (17.1)	557				
	<i>E. coli</i>	45 (4)	59 (4.5)	24 (2.7)	128				
	<i>P.mirabilis</i>	28 (2.6)	22 (1.8)	24 (2.7)	74				
	<i>K. oxytoca</i>	3 (0.3)	3 (1.1)	14 (1.6)	20				
	<i>C.freundii</i>	3 (0.3)	7 (0.5)	6 (0.7)	16				
Gram positive bacteria (n=75)	<i>S. aureus</i>	19 (1.8)	35 (2.6)	13 (1.5)	67	22	38	15	75
	<i>E. faecalis</i>	3 (0.3)	3 (0.2)	2 (0.2)	8				
Fungi (n=9)	<i>Candida albicans</i>	3 (0.3)	4 (0.3)	2 (0.2)	9				

Overall results of susceptibility testing are shown in Table 2. Among gram-negative bacteria *Acinetobacter baumannii* showed overall >90% resistance for ceftazidime, amikacin and ciprofloxacin with no significant change. There was significant increase in imipenem, meropenem and piperacillin/tazobactam resistance in 2014 (82%, 95%, 92% respectively) in comparison to 2013 (48%, 72%, 79% respectively) and 2012 (36%, 64%, 55% respectively). There was significant decrease in cefoperazone/sulbactam resistance in 2013 (11%) in comparison to 2012 (25%) and increase in 2014 (25%) in comparison to 2013(11%). *Pseudomonas aeruginosa* showed overall >50% resistance for ceftazidime, meropenem and ciprofloxacin with no significant change. There was significant decrease in amikacin and netilmicin resistance in 2014 (52%, 51% respectively) in comparison to 2012 (67%, 70% respectively) with no significant change in 2013 (65%, 63% respectively). There was significant decrease in imipenem and cefoperazone/sulbactam resistance in 2013 (25%, 18% respectively) in comparison to 2012 (40%, 37% respectively) and increase in 2014 (45%, 31% respectively) in comparison to 2013. Resistance to piperacillin/tazobactam was significantly decreased in 2013 (17%) in comparison to 2012 (30%) with no significant change in 2014.

*Klebsiella pneumoniae* showed overall >80% resistance for amikacin, netilmicin and ciprofloxacin with no significant change. There was significant increase in imipenem, cefoperazone/sulbactam and piperacillin/tazobactam resistance in 2014 (37%, 55%, 78% respectively) in comparison to 2012 (13%, 25%, 44% respectively) with no significant change in 2013 (22%, 24%, 53% respectively). There was significant increase in ertapenem and meropenem resistance in 2013 (62%, 54% respectively) and 2014 (75%, 59% respectively) in comparison to 2012 (39%, 30% respectively). Resistance to

*Proteus mirabilis* showed overall >75% resistance for ceftazidime with no significant change. Resistance to amikacin and netilmicin was significantly decreased in 2014 (64%, 71% respectively) in comparison to 2013 (83%, 87% respectively) and 2012 (82%, 88% respectively) with no significant change in 2012 and 2013. There was significant increase in imipenem resistance in 2014 (25%) in comparison to 2012 (5%) and 2013 (5%). There was significant increase in meropenem resistance in 2013 (67%) in comparison to 2012 (27%) and 2013 (25%). Resistance to ciprofloxacin was significantly increased in 2014 (91%) and 2013 (91%) in comparison to 2012 (75%). No resistance was observed for cefoperazone/sulbactam and piperacillin/tazobactam in 2013 which was significant in comparison to 2012 (30%, 30% respectively) and 2014 (29%, 22% respectively).

*Klebsiella oxytoca* showed overall 100% resistance for ciprofloxacin. Resistance to ceftazidime, ertapenem, meropenem and cefoperazone/sulbactam was significantly increased in 2013 (100% for all) and 2014 (83%, 67%, 70%, 67% respectively) in comparison to 2012 (67%, 33%, 33%, 33% respectively) with significant decrease in 2014 in comparison to 2013. There was significant increase in resistance to piperacillin/tazobactam and amikacin in 2013 (100% for both) in comparison to 2012 (67% for both) with significant decrease in 2014 (60%, 80% respectively) in comparison to 2013. No resistance was observed for imipenem in 2013 which was significant in comparison to 2012 (33%) and 2014 (40%). Gram-negative bacteria showed overall >70 % resistance to ceftazidime, amikacin, netilmicin and ciprofloxacin with no significant change over three years. Resistance to meropenem and piperacillin/tazobactam was significantly increased in 2014 (73%, 68% respectively) in comparison to 2012 (53%, 45% respectively).



Resistant to imipenem was significantly increased in 2014 (60%) in comparison to 2013(35%) and 2012 (31%). Resistance to cefoperazone/sulbactam was significantly decreased in 2013 (16%) in comparison to 2012 (29%) with significant increase in 2014 (33%) in comparison to 2013.

Among gram positive bacteria *Staphylococcus aureus* showed >95% resistance to penicillin, >60% to erythromycin, >75% to ciprofloxacin with no significant change in three years. MRSA detection rate was >60% with no significant change. Resistance to gentamicin was significantly high in 2013(84%) in comparison to 2012(57%) and 2014(62%). Clindamycin resistance was significantly increased in 2013 (77%) in comparison to 2012 (50%) with significant decrease in 2014 (22%) in comparison to 2013 and 2012. None was resistant to vancomycin. Among *Enterococcus faecalis* penicillin resistance was significantly increased in 2013 (100%) in comparison to 2012 (67%) with significant decrease in 2014 (50%) in comparison to 2013 and 2012. Ciprofloxacin resistance was significantly increased in 2013 (100%) and 2014 (50%) in comparison to 2012 (33%) with significant increase in 2014 in comparison to 2012. 67% isolates were resistant to high gentamicin in 2012 and 2013 with no resistance in 2014. VRE were detected only in 2012(33%).

Two or more than two samples were received from 501(159, 208, 134 in 2012, 2013, 2014 respectively) patients out of 1232 patients. Of the 159, 208, 134 first cultures, 137(86.2%), 171 (82.2%), 117 (87.3%) had a single microbe, 22 (13.8%), 37 (17.8%), 15 (11.2%) had two in 2012, 2013, 2014 respectively. Of the 159, 208, 134 second cultures, 128(80.5%), 151(72.6%), 93 (69.4%) had a single microbe, 31 (19.5%), 56 (26.9%), 41 (30.6%) had two in 2012, 2013, 2014 respectively. In 2013, 1 (0.5%) had three microbes and in 2014, 1 (0.7%) had three, 1 (0.7%) had four microbes. The median interval between cultures pairs was 4.5 days, 4 days and 4 days in 2012, 2013 and 2014 respectively with no significant difference. Most adults had a change in flora in their tracheal aspirate cultures in the culture pairs (Table 3). Both loss and gain of species occurred. New species were present in 66%, 62% and 67.9% of second cultures in 2012, 2013 and 2014 respectively. It was observed that in spite of no change in flora in first and second cultures antimicrobial susceptibility patterns often differed between the isolates, suggesting acquisition of new resistance capacities. Additional antibiotic coverage was needed in 46.5%, 44.2%, 55.2% patients in 2012, 2013 and 2014 respectively with no significant difference. No associations were identified between (1) any changes in microbial species, (2) any gain of microbial species, or (3) need for additional antibiotics to effectively treat the microbe(s) isolated in the second culture over the years. Overall results of culture pairs are shown in Table 3.

## DISCUSSION

Health care associated infections are a serious concern. Hospitalized patients especially in ICU are seriously ill or due to age or immunological status are more prone to get infections. Infections are among the most important and the leading cause of mortality and morbidity in ICU. Endotracheal tubes are susceptible to infection and therefore it is important to be aware of the relevant factors and responsible

organisms to take prompt action. The findings of this study would be helpful in selection of appropriate antibiotics. In this study, Culture of 5319 samples of tracheal aspirate was done during 2012 to 2014. A total of 3268 (1063 in 2012, 1317 in 2013, 886 in 2014) isolates were recovered from 1232 patients (392 in 2012, 533 in 2013 and 307 in 2014). Positive culture rate was 55%. The study by Simoni *et al.* showed that 100% of samples from airway prosthesis are positive in culture (Simoni *et al.*, 2004). Cardinosa *et al.* have reported a positive culture result in 89% of their samples (Cardinosa *et al.*, 1999).

The variation could be explained by the technique of intubation, clinical and individual characteristics of study population, colonization during intubation or lack of sufficient precautions for intubation due to the high work load in an emergency setting (Oncag *et al.*, 2005). In this study, *Acinetobacter baumannii* was the most prevalent isolate followed by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* with relatively high antibiotic resistance. Amini *et al.* also conducted a study on distribution of isolated microorganisms from tracheal tube of ICU patients declaring that *S. aureus* (23.6%), *Klebsiella* spp. (23.3%), *Acinetobacter* spp. (20.7%), *P. aeruginosa* (18.2%), *E. coli* (7.7%), and *Enterobacter* spp., were the most common isolates (Amini *et al.*, 2009). Abdollahi *et al.* study showed that *Acinetobacter* spp., *P. aeruginosa*, and *S. aureus* are among the most 3 prevalent isolated organism from endotracheal tube (Abdollahi *et al.*, 2013). There was no significant change in the colonizing flora over the years. Among gram negative bacteria about one third organisms were resistant to imipenem and half of them were resistant to meropenem which is also an alarming sign. Colistin resistant was not detected. Among gram-positive bacteria *Staphylococcus aureus* was most commonly isolated with >60% MRSA rates.

Surveys of the prevalence and antibacterial susceptibility patterns of bacterial isolates are important for determining appropriate empirical therapy for infections in critically-ill patients. Also, epidemiological analysis of patient data can be informative for appropriate management of patients in ICUs. The prescribing of antibiotics in the ICU is usually empiric. Therefore, the ongoing surveillance of antibiotic susceptibility patterns of predominant bacteria is a fundamental effort to monitor changes in susceptibility patterns and to guide the clinician in choosing empirical or directed therapy appropriately. Appropriate antibiotic utilization in ICU is crucial not only in ensuring an optimal outcome, but also in preventing the emergence of multi drug resistance bacteria. Therefore, developing nationwide antibiotic policy and guidelines is essential to limit multidrug resistance and to maintain low level of resistance to newer antibiotics. Our hospital has its own hospital antibiotic policy.

The present study compared 501 culture pairs with a time interval of around 4 days and evaluated cultures obtained during ICU stay. Change in flora was present in around 72% patients. Additional antibiotic coverage was needed in around 50% (45%-55% over 3 years) of the patients. The increased variability of flora and the potential need to adjust antibiotic coverage based on culture data obtained during exacerbations suggest that surveillance tracheal aspirates are of important in management. This study has certain limitations. One of the

limitations of this study was the fact that although antibiotic resistance pattern is important to intensive care physician, this is also heavily influenced by the antibiotic usage pattern in the study patients prior to obtaining the sample and also overall antibiotic usage pattern. Information regarding health-care-related events, antimicrobial exposures, and other factors between the first and second cultures that may have led to alterations in microbial flora was not uniformly available. Also, whether a particular isolate in a culture represented colonization or pathogen was not always possible to assess. In conclusion, this study demonstrates large drift over time in bacterial flora and their antibiotic sensitivity pattern. Routine cultures would enable the physician to monitor changes in flora and make appropriate antibiotic selections during exacerbations.

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