

ANTIBIOGRAM PROFILE OF BACTERIA COLONIZING THE ENDOTRACHEAL TUBES (ETTS) OF PATIENTS ADMITTED TO INTENSIVE CARE UNITS (ICUS) IN A TERTIARY CARE HOSPITAL OF NEPAL

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ABSTRACT

Hospital acquired infection (HAI) in intensive care units (ICU) are responsible for high morbidities and mortalities worldwide due to emergence of resistant bacteria. In developing countries, due to lack of knowledge of proper surveillance, proper resources and proper guidance this burden was somewhat underestimated. Thus, the aim of this study was to look for the spectrum of bacteria colonizing the ETTs and to determine proper empirical antibiotic therapy. We collected endotracheal tube aspirates from 188 patients of admitted in ICU of Manipal Teaching Hospital, Pokhara. All bacteria were identified by conventional techniques. Antimicrobial sensitivity testing was performed on Mueller-Hinton agar plates with commercially available antibiotic discs using Kirby-Bauer disc diffusion techniques and interpreted as per the guidelines of CLSI. The antibiotic discs (conc.) used were: piperacillin/tazobactam (100/10mcg), ciprofloxacin (5mcg), amikacin (30mcg), imipenem (10mcg), gentamicin (10mcg), cefaperazone sulbactam(75/10mcg), for Gram negative bacteria and erythromycin (15mcg), amikacin (30mcg), gentamicin (10mcg), ciprofloxacin (5mcg), and clindamycin (2mcg) for Gram positive bacteria. A total of 188 ETTs investigated, 128(68.08%) yielded positive culture. Single type of organisms was found in 119 (63.29%) and 9 (4.7%) cases yielded mixed type of growth. *Acinetobacter* spp. were the most predominant organism among all gram-negative organisms, which was found to be in 71 (51.82%) cases, followed by *Klebsiella pneumoniae* in 27 (19.7%), *Pseudomonas aeruginosa* in 23 (16.78%), *Escherichia coli* in 5 (3.64%), *Enterobacter* in 2 (1.46%). Whereas, *Staphylococcus aureus* (4.37%) was the commonest among all gram-positive organism followed by coagulase negative *Staphylococcus* in 2 (1.46%) and *Enterococcus* in 1 (0.73%). Most of *Acinetobacter* spp. showed resistance to ciprofloxacin (84.5%), while 74.6% were resistant to amikacin, 73.2% to gentamicin, 71.83% to piperacillin-tazobactam and 42.2% towards imipenem. Out of the 6 strains of *S. aureus*, 5 (83.3%) were methicillin resistant. Due to the increasing incidence of organisms in ICUs, an early and correct diagnosis of ETT associated infections is a challenge for optimal antibiotic therapy. Therefore, the best approach to manage the respiratory infections following ETT application will be appropriate use of antibiotics with adaptation of proper infection control measures, which could help to prevent further spread of infection.

KEYWORDS

Hospital acquired infection, Endotracheal Tubes, Colonizing bacteria, Intensive Care Units

Received on:

Accepted for publication:

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DOI: <https://doi.org/10.3126/nmcj.v23i3.36317>

INTRODUCTION

Hospital-acquired infection (HAI) is a serious problem worldwide.¹ It was shown that in developed countries 5–15% patients in general wards and 50% patients in intensive care units (ICU) suffered from HAI. In developing countries this burden was somewhat underestimated, which might be due to lack of knowledge of surveillance, proper resources and proper guidance.²

According to a study done by WHO, the highest prevalence of nosocomial infections occurs in intensive care units, surgical and orthopaedic wards. Infection rates are higher among patients with increased susceptibility because of old age, underlying diseases like diabetes and depressed immunity, or chemotherapy.³ The indwelling medical devices are responsible for predisposition of HAIs that include endotracheal tubes, catheters, and different surgical appliances. At the same time, indwelling medical devices are the major tools in the clinical management of hospitalized patients, particularly those who require life-supporting measures. These devices are applied to more than 25% of hospitalized patient and perpetually act as bridge between the nonsterile outside environment and the sterile in vivo environment of the patient.⁴ Medical device infections are therefore, quite often linked to colonization of devices by microbes.⁵ Invasive medical procedures in the intensive care units remarkably increase the risk of such infections.

Ventilator associated pneumonia (VAP) and catheter associated urinary tract infections have been shown to be the greatest risk to patient's safety. Moreover, the use of different kinds of catheters, endotracheal tubes, supplying apparatuses and surgeries are the most common ways for transmission of nosocomial infections.^{4,6} The most frequent nosocomial infections are those of surgical wounds, urinary tract infections and lower respiratory tract infections.^{7,8}

Among these, central line associated blood stream infections, catheter-associated urinary tract infections, surgical site infections and ventilator-associated pneumonias are the most frequently encountered ones seen in our setting. Many different pathogens may cause such infections.³ These include Gram-positive bacteria like *Staphylococcus aureus* and Gram-negative bacteria like *Escherichia coli*, *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp.

Bacterial colonization of the pharynx and upper respiratory tract is the initial portal of

entry into normally sterile lower respiratory tract. Impaired physiological defense mechanisms including the cough reflex and innate immune system can predispose the airway to microbial invasion.⁹ Colonization is enhanced by therapeutic measures like endotracheal intubation and suction.¹⁰ The surface of an endotracheal tube (ETT) provides bacteria with a substratum that promotes microbial colonization and biofilm formation. The formation of biofilm around the ETT by the microorganisms¹¹ and their subsequent dislodgement following endotracheal suction and repeated intubations contributes to lung colonization and may lead ultimately to VAP. Intubation with mechanical ventilation increases the risk of pneumonia 6 to 20 folds more among patients and is associated with crude mortality rates of 20% to 40%.^{12,13}

Despite the afore mentioned advances in the knowledge regarding the etiopathogenesis of VAP, there are scanty reports on the different types of bacteria colonizing on the indwelling devices in the respiratory tract, especially on the ETTs, which are frequently used as life supporting measures in ICU patient in our setting. Thus, the aim of this study was to look for the spectrum of bacteria colonizing the ETTs isolated from the ETT aspirates and to determine the antibiotic susceptibility patterns of these isolates.

MATERIALS AND METHODS

A total of 188 ETTs obtained from the patient admitted in Manipal Teaching Hospital, Pokhara, Nepal between September 2019 and January 2021 were studied. Only those ETTs, which were in place for more than 48 hours, were included. Tips of ETTs approximately two inches from the distal end were cut using sterile techniques and placed in a sterile bottle and sent to the microbiology laboratory. For culture of the tip, Maki's roll-plate technique was used. Growth of 15 or more colonies were considered as significant.¹⁴

In addition, an attempt was made to isolate the bacteria colonizing the intraluminal surface of the ETTs. For this, the tips were suspended in sterile phosphate buffered saline and vortexed. The washed-out fluid was cultured on blood agar and MacConkey plates. An yield of $>10^5$ colony forming units (CFU)/mL of the vortexed specimen was taken as significant. All bacteria were identified by conventional techniques.^{15,16} Antimicrobial sensitivity testing was performed on Mueller-Hinton agar plates with commercially available antibiotic discs (Hi-media, Mumbai, India) using Kirby-Bauer

disc diffusion technique¹⁷ and interpreted as per the guidelines of CLSI. The antibiotic discs (concentration) used were: piperacillin/tazobactam (100/10mcg), ciprofloxacin (5mcg), amikacin (30mcg), imipenem (10mcg), gentamicin (10mcg), and cefaperazone sulbactam(75/10mcg) for Gram negative bacteria and erythromycin (15mcg), amikacin (30mcg), gentamicin (10mcg), ciprofloxacin (5mcg), and clindamycin (2mcg) for Gram positive bacteria. *S. aureus* resistant to ceftazidime (30 mcg) were considered as methicillin resistant *S. aureus* (MRSA). Data were analyzed by descriptive statistics using SPSS version 18.

RESULTS

Table 1: Bacteria isolated from endotracheal tubes.

Organism	Culture positive (n=128) No. of organism isolated (%)
Single type of growth	
<i>Acinetobacter</i> spp	67 (52.34)
<i>Ps. aeruginosa</i>	14 (10.9)
<i>K. pneumoniae</i>	22 (17.1)
<i>E. coli</i>	5 (3.9)
<i>Enterobacter</i> spp	2 (1.56)
<i>S. aureus</i>	6 (4.68)
Coagulase negative <i>Staphylococcus</i>	2 (1.56)
<i>Enterococcus faecalis</i>	1 (0.78)
Mixed type of growth	
<i>Ps. aeruginosa</i> + <i>Acinetobacter</i> spp	4 (3.1)
<i>Ps. aeruginosa</i> + <i>Klebsiella</i> Spp	5 (3.9)
Total	128 (100)

From a total 188 specimens investigated, 128 (68.08%) yielded positive culture. The majority of the organisms were obtained from patients in age group of 61 to 75 years (50; 27.2%). Single type of organism was found in 119 (63.29%) cases and 9 (4.7%) cases yielded mixed type of growth (Table 1).

As shown in Table 1, out of all gram-negative organisms isolated; *Acinetobacter* spp. was the most predominant organism, being obtained in 71 (51.82%) cases, followed by *K. pneumoniae* in 27 (19.7%), *Ps. aeruginosa* in 23 (16.78%), *E. coli* in 5 (3.64%), *Enterobacter* spp. in 2 (1.46%). However, *S. aureus* (6/137; 4.37%) was the commonest among all gram-positive organism followed by coagulase negative *Staphylococcus* in 2 (1.46%) and *Enterococcus* in 1 (0.73%).

As depicted in table 2, Most of the *S. aureus* isolates were resistant to erythromycin, clindamycin and ciprofloxacin (83.3%, 83.3% and 83.3% respectively). The percentage of resistance to amikacin and gentamicin were found to be 50% in each case. Out of the 6 strains of *S. aureus*, 5 (83.3%) were methicillin resistant. coagulase negative *Staphylococci* (n=2) were observed to be resistant to ciprofloxacin (100%) and erythromycin (100%). Out of the 2 strains of coagulase negative *Staphylococci* (n=2), only one was methicillin resistant.

Majority (84.5%) of *Acinetobacter* spp. showed resistance to ciprofloxacin, while 74.6% were resistant to amikacin, 73.2% to gentamicin, 71.83% to piperacillin-tazobactam, 69.01% to cefaperzone sulbactam and 42.2% to imipenem.

A total of 66.6% of *K. pneumoniae*, were resistant to ciprofloxacin, 44.4% to amikacin, 48.1% to gentamicin, 51.8% to piperacillin-tazobactam, 44.4% to cefaperazone sulbactam and 40.7% to Imipenem (Table 3).

Table 2: Resistance pattern of Gram-positive isolates.

Name of antibiotics	<i>S. aureus</i> (n=6) No. of resistant/total no of isolates (% resistance)	Cogulase negative <i>Staphylococcus</i> (n=2) No. of resistant/total no of isolates (% resistance)	<i>Enterococcus</i> (n=1) No. of resistant/total no. of isolates (% resistance)
Erythromycin (E)	5 (83.3)	2 (100)	1 (100)
Clindamycin (CD)	5 (83.3)	---	---
Ceftazidime (Cx)	5 (83.3)	1 (50)	--
Amikacin (AK)	3 (50)	0 (0.0)	0 (0.0)
Gentamicin (GEN)	3 (50)	0 (0.0)	0 (0.0)
Ciprofloxacin (CIP)	5 (83.3)	2 (100)	---

Number in parenthesis indicate percentage

Table 3: Resistance pattern of Gram-negative isolates.

Name of antibiotics	<i>Acinetobacter</i> (n=71) No of resistant / total no. of isolates (% resistance)	<i>K. pneumoniae</i> (n=27) No of resistant/total no. of isolates (% resistance)	<i>Ps. aeruginosa</i> (n=23) No of resistant/total no. of isolates (% resistance)	<i>E. coli</i> (n=5) No of resistant/ total no. of isolates (% resistance)	<i>Enterobacter</i> (n=2) No of resistant/ total no. of isolates (% resistance)
Amikacin (AK)	53 (74.6)	12 (44.4)	3 (13.04)	5 (20)	1 (50)
Gentamicin (GEN)	52 (73.2)	13 (48.1)	3 (13.04)	1 (20)	1 (50)
Piperacilin tazobactam (PIT)	51 (71.83)	14 (51.8)	6 (26.08)	1 (20)	1 (50)
Imipenen (IPM)	30 (42.2)	11 (40.7)	2 (8.6)	1 (20)	0 (0.0)
Ciprofloxacin (CIP)	60 (84.5)	18 (66.6)	8 (34.78)	2 (40)	1 (50)
Cefaperazone sulbactam (CFS)	49 (69.01)	12 (44.4)	3 (13.04)	0 (0.0)	0 (0.0)

Resistance to Ciprofloxacin was seen among 34.78% of the *Ps. aeruginosa* isolates. However, 13.04% of them showed resistance to amikacin 13.04% to gentamicin, 26.08% to piperacillin-tazobactam, 13.04% to cefaperzone sulbactam and 8.6% to imipenem (Table 3). All *E. coli* were sensitive to cefaperazone sulbactam. All *Enterobacter* spp. were sensitive to imipenem and cefaperazone sulbactam (Table 3).

DISCUSSION

Nosocomial infections of bacterial origin are on an increasing trend, especially those happening among ICU patients on indwelling medical devices.¹⁹ It is postulated that persistence of bacteria is related to their capacity to colonize the devices by way of production of biofilm. The present study documented prevalence of nosocomial pathogens among patients on ETT.

From a total of 188 investigated specimens, 128 (68.08%) had positive culture in our study. Culture positivity was seen in 123 (65.4%) males and 65 (34.6%) females. In a similar study done by Panda *et al.*,¹⁸ it was found that 70% had significant growth and occurrence of Ventilator associated pneumonia (VAP) was common in men (64%) than in women (36%).

In this study, *Acinetobacter* spp. was the most prevalent organism, being obtained in 71 (51.82%) cases, followed by *K. pneumoniae* in 27 (19.7%), *Ps. aeruginosa* in 23 (16.78%), *E. coli* in 5 (3.64%), *Enterobacter* spp. in 2(1.46%). In a similar study conducted by George *et al.*,¹⁹

Acinetobacter spp. accounted for 37.5% of the isolates and were the most common organism isolated followed by *Ps. aeruginosa* (21.8%) and *K. pneumoniae* (15.6%). Panda *et al.*¹⁹ also found that *Acinetobacter* spp. were the most common organism causing VAP, followed by *Pseudomonas* species.

In yet another study, Patil *et al.*,²⁰ observed that *Ps. aeruginosa*, was the most common isolate from ETT, followed by *K. pneumoniae*. In contrast, Rello *et al.*²¹ in their study demonstrated that *P. aeruginosa* was the most common causative organism in infection related to ETT and this could be the result of large number of their subjects being patients of chronic obstructive pulmonary disease (COPD) or being under intubation or having a previous history of antibiotic therapy.

Although *Acinetobacter* spp. are comparatively less virulent than *Pseudomonas* spp. yet, there is a recent trend of more and more resistance among clinical isolates of *Acinetobacter* spp. from the present study to the commonly used antimicrobial agents.⁵ It was noteworthy that 42.2% of the *Acinetobacter* spp. and 40.7% of *K. pneumoniae* isolates were resistant to imipenem. Out of 71 isolates of *Acinetobacter* spp. (30/71; 42.2%) was found to be multidrug resistant, which is quite alarming because carbapenems have been the antibiotic of choice against multidrug resistant bacteria in ICU patients and a recent report of emergence of carbapenem resistance among *Acinetobacter* spp. isolates remains a therapeutic challenge.²²

Thus, there are limited options for the treatment of infections in ICU patients due to multidrug resistant (MDR) *Acinetobacter* spp. especially due to those showing resistance to carbapenems. In such a scenario, tigecycline and colistin happen to be the drugs of choice. A recent study from India 23 showed very low rate of resistance (10.69%) towards tigecycline exhibited by *Acinetobacter* spp. In contrast to this, the present study depicted that 100% of *Acinetobacter* spp. irrespective of their carbapenem susceptibility were sensitive to tigecycline. Besides, all *Acinetobacter* spp. and *Pseudomonas* spp. were sensitive to colistin too. This is in contrast to the Mostafa *et al* observations 24 who reported colistin resistance among 10.78% of their isolates, which was attributed to frequent use of colistin in their clinical practice. In our set up, judicious use of both colistin and tigecycline could be the reason that all our clinical isolates of *Acinetobacter* spp. were sensitive to these two drugs.

In our study, *S. aureus* (6/137; 4.37%) was found to be the commonest among gram-positive organisms followed by Coagulase Negative *Staphylococcus* (2; 1.46%) and *Enterococcus* (1; 0.73%). We found that 5 out of 6 *S. aureus* strains were methicillin resistant. In a similar study done by Swati *et al*, it was noticed that out of the total 7 isolates of *S. aureus*, 6 (86%) were MRSA. 25 In another study Veena Krishnamurthy *et al*.²⁶ found that prevalence of MRSA among ET aspirates was 18.15%. Vandecandelare *et al*²⁷ however, observed that 4 out of 7 of their *Staphylococcus aureus* biofilm positive isolates from ET cultures were methicillin resistant. Garland *et al*⁹ and Aly *et al*¹⁰ postulated the role of microbiome biofilms responsible for drug resistance. They were of the view that biofilm was formed on the ETT, soon after intubation, which contributed to the development of VAP because aggregates of ETT biofilm can easily be dislodged by means of suction of catheters toward the lower respiratory tract.^{10,28}

Biofilm formation and methicillin resistance *vis a vis* multidrug resistance were investigated in the past on *S. aureus* and *S. epidermidis* isolates in indwelling device related infections.¹² Occurrence of methicillin/oxacillin resistance and multidrug resistance were reported to be higher among biofilm producing organisms as compared to non-biofilm producing organisms.

Hence, as emphasized previously bacteria embedded in the interior of biofilms were protected from antibiotics or could be adopting mechanism of acquiring drug resistant genes in their sessile mode of existence; thus accounting

for the observable *in vitro* resistance. Nevertheless, such high-level drug resistance has definite impact on the therapeutic outcome and patient management. Though our study has the limitation that, these ETT isolates were not studied for their ability to form biofilms yet it could well be derived that upon colonization onto ETT, majority of them could have the potential to form biofilms on those devices, as evidenced by the exhibition of drug resistance especially multidrug resistance among these isolates. Recent studies showed increasing incidence of MDR pathogens among patients with VAP.^{29,30}

Due to the increasing incidence of nosocomial infection in ICUs patient on indwelling medical devices, an early diagnosis of ETT associated infections is a challenge. With increased duration of intubation leads to colonization onto ETT which have a potential to form biofilm on those devices. ETT colonization with biofilm-producing organisms increased the risk of developing VAP with highly resistant strains. Hence, we recommend that use of appropriate antibiotic with adaptation of infection control measures among ICU patient with indwelling devices which can have enormous impact on management as well as reduces stay in hospital.

Limitation: Minimal inhibitory concentration of these drug were not performed.

Conflict of Interest: None

Source of Research Fund: None

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