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Microbiological profile and anti-microbial susceptibility pattern of isolates from endo tracheal aspirate and endo tracheal tube tips in ICU patients: A comparative study

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***Abstract*---**Background: There has been a surge of sufferers contracting healthcare-acquired infections (HAIs) in recent times. This has been further exacerbated by the SARS-CoV-2 pandemic which, beginning in 2020, has severely affected the majority of the world's

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nations, and one of the biggest problems caused by this infection was the high number of patients who required critical care. Aim: We sought to identify the microbiological profile and antimicrobial susceptibility pattern of bacteria isolated from tracheal aspirate and endotracheal tube (ETT) tips of patients who had to undergo ICU admittance. Methodology: We isolated a total of 150 microbial samples from 131 patients, of which 97 came from endo tracheal aspirates and the remaining 53 samples were obtained from ETT tips, following which the isolation and identification of bacteria was then done according to requisite microbiological assays. The Kirby-Bauer Technique with Mueller-Hinton agar was used to conduct the antimicrobial susceptibility testing in accordance with Clinical and Laboratory Standards Institute (CLSI) 2016 standards. Results: 53 (35%) of the 150 total specimens were from ETT tips, whereas 97 (65%) came from tracheal aspirates. 101 (67%) of the organisms were gram-negative, and 49 (33%) were gram-positive. Conclusion: As a result of bacterial colonisation, patients admitted to the ICU are substantially more likely to develop respiratory tract infections. Multi-drug resistance bacteria are brought on by the improper use of broad-spectrum antibiotics.

***Keywords*---**antibiotics, endo tracheal aspirates, ETT tips, gram staining, ICU, microbial invasion**.**

Introduction

A deregulated host response to infections results in sepsis, a life-threatening disease accompanied by physiological, pathological, and molecular abnormalities. Due to its high death and morbidity rates as well as its significant economic cost, it is a global public health concern. [1] Rudd and colleagues recently published the startling estimates of 48.9 million sepsis cases and 11.0 million sepsis-related deaths worldwide in 2017. [2] More than 30 million cases of hospital-treated sepsis are thought to occur annually globally, with 5.3 million individuals passing away from sepsis, according to a systematic review that was published in 2016 and was based on studies from high-income nations. [3]

Sepsis also plays a significant role in the intensive care unit (ICU), where it affects about 30% of patients, albeit there are significant regional differences. According to a US study involving more than 170,000 sepsis patients, ICU admission was necessary in 55% of all sepsis cases. Sepsis affects people of all ages, but new-borns are particularly vulnerable to illness. [4]

According to Reinhart and colleagues, sepsis cases that are complications of illnesses picked up in the community account for up to 70% of all instances of sepsis. It may also result from infections linked to healthcare settings (HAIs), which may often be avoided by taking the proper steps for infection prevention and control (IPC). [5] The World Health Organization (WHO) reported in 2011 that the prevalence of HAIs ranged between 5.7 and 19.1 percent hospital-wide. According to more current data, the prevalence of HAIs across all hospitals in

Europe and the USA is 6.5 percent and 3.2 percent, respectively. HAIs were shown to be the cause of 60% of sepsis cases in ICUs in Brazil, according to a multicentre prospective study. This finding suggests that HAIs are more of a burden on the epidemiology in low- and middle-income nations. [6]

Importantly, recent data revealed that the deployment of multiple IPC interventions can prevent up to 55% of all HAIs, which would ultimately lead to a marked decline in the number of cases of hospital-acquired sepsis (HA sepsis). [7] There hasn't been a thorough study of the worldwide burden of HA sepsis yet, including in the ICU environment, and the majority of sepsis studies fail to distinguish between community-acquired and HA sepsis. [8]

Nosocomial infections have become a serious threat to mortality and morbidity among hospitalised patients. [9] The World Health Organization (WHO) reports that intensive care units (ICUs) have the greatest percentage of nosocomial infections, which is 5 to 7 times greater than other settings. [10] Defective immune systems and excessive invasive device use are the main risk factors among critically ill patients. Infections linked to medical devices, like ventilator-associated pneumonia (VAP), are more common in underdeveloped nations. VAP is responsible for 24-50 percent of mortality on average, with potential increases of up to 76 percent depending on the environment and host-pathogen connection. [11]

The duration of the hospital stay, exposure to the intensive care unit (ICU), usage of invasive procedures, and improper or prolonged exposure to wide spectrum antibiotics are some risk factors that can encourage multi-drug resistant (MDR) microorganisms to infect hospitalised patients. [12] Endotracheal tube use and tracheal incubation can both prevent certain components of innate immunity from functioning properly. [13] The aetiology of VAP is heavily influenced by the presence of biofilm on ETT and pathogenic organism aspiration from upper respiratory tract. [14] The use of ETT raises the risk of developing pneumonia because it encourages the build-up of tracheobronchial secretions by reducing mucocilliary clearance and interfering with the cough reflex. [15] The insertion of ETT also makes it easier for indigenous and foreign microorganisms to harm and colonise tracheal mucosa. Additionally, ETT, which functions as a source of a bridge between the oropharynx and trachea, is a key factor in the development of bacteria in the respiratory tract. [16] Because it promotes bacterial growth by establishing a microenvironment that prevents bacteria from accessing antibiotics, the presence of biofilm in the lumen of ETT also poses a serious risk for the development of antibiotic resistance. [17] The etiologic agents vary depending on a number of variables, including the kind of ICU, previous use of antibiotic therapy, and pre-existing disease. According to numerous studies, gram-negative bacteria are the primary cause of more than 30% of hospital acquired infections and more than 40% of infections in ICU patients. [18]

Due to the widespread use of broad-spectrum antibiotics, antibiotic resistance among these ICU infections poses a significant additional hazard. [19] The misuse and abuse of antibiotics are the global drivers of resistance in this hospital acquired pathogens. [20] According to reports, using more -lactam medications can make bacteria resistant to these antimicrobials, and by creating -lactamases, they

can become resistant to a variety of -lactam antibiotics. [21] Clinical practitioners faced a significant difficulty because there are few effective treatments available to combat the infection brought on by these MDR bacteria.

The primary aim of our comparative study was to identify the frequency and antibiotic susceptibility of organisms isolated from tracheal aspirate and ETT specimens and measuring their performance against commonly used antibiotics in clinical settings, which will aid doctors in selecting the best antimicrobial treatment for these MDR bacteria and preventing life-threatening infections.

Materials and Methods

Study hypotheses

Through the means of this study, we aimed to investigate the various microbial species that were present in our study subjects who were admitted to the ICU through the means of their endo tracheal aspirates and their ETT tips.

Study design

We aimed to perform a comparative study of the various microbial specimens that we obtained, analysing their percentage of incidence in our study subjects, followed by observing their performance against the antibiotics/anti-microbial drugs and understanding whether our samples were respondent or resistance to them.

Sample size and sampling

We selected 131 patients admitted to the ICU section of the Hitech Medical College and Hospital, Bhubaneswar, Odisha, who were undergoing treatment and analysed their endo tracheal aspirates and ETT tips, by the means of which we were successful in ultimately obtaining 150 microbial samples. Out of these 150, 97 specimens were obtained from endo tracheal aspirates, with the remaining 53 being isolated from ETT tips. The isolates were identified based on cultural traits, appearance, and biochemical profiles. Prior to choosing the sample, proper labelling of the specimen in a sterile container was also noticed.

Inclusion/exclusion criteria

With respect to the samples obtained for our study, we did not limit the selection criterion to variables such as age, gender, demographic characteristics or the systemic condition/illness of the patient. Patients who were discharged/admitted to the ICU very recently, or had to undergo a major surgical procedure within a short period of time were excluded from the domains of our study. Also, patients who had to be hooked up to a ventilator or were too debilitated were not considered for the sampling.

Laboratory procedures

All samples were maintained on Blood and MacConkey agar and kept overnight at 37°C in an aerobic environment. The identification of an organism from culture media as Gram-positive or Gram-negative was done using the Gram staining technique. Then, basic biochemical tests such as catalase, coagulase, and oxidase assays were carried out for identification. Analytical Profile Index was used to further corroborate the identification of the bacteria species. The Kirby-Bauer Technique with Mueller-Hinton agar was used to conduct the antimicrobial susceptibility testing in accordance with Clinical and Laboratory Standards Institute (CLSI) 2016 standards. Zone diameter was measured in millimetres and interpreted in accordance with CLSI criteria (mm).

Again, as per CLSI recommendations, antimicrobial susceptibility testing was carried out using the Kirby-Bauer disc diffusion method. The following drugs were utilised in the study: Amikacin (30μg), Gentamicin (10μg), Doxycycline (30μg), Ciprofloxacin (5μg), Ceftriaxone (30μg), Penicillin (10units), Oxacillin (30μg), Vancomycin (30μg), imipenem disc (10 g) and piperacillin-tazobactam (10 g/100 g). From an isolated colony of pathogens chosen from 18–24-hour agar plates, a Mueller–Hinton broth (MHB) inoculum of 0.5 McFarland standards turbidity was generated. A sterile cotton swab was put into the inoculum suspension after 15 minutes. The Mueller-Hinton agar (MHA) plate's dry surface was inoculated by streaking the swab over it after the swab had been rotated several times and pressed firmly against the interior wall of the tube above the fluid level. The swab was streaked over the agar surface two more times at 60° to ensure a uniform distribution of inoculum. Antibiotic discs were inserted and pushed down to establish complete contact with the agar surface after 15 minutes. To guarantee a minimum of 24 mm between the centres, the discs were dispersed uniformly. Following the application of the disc, the plates underwent a 16–18-hour aerobic incubation period at 37 °C within 15 minutes. The antibiotics and the media used are from Tulip Diagnostics Private Limited, and MICROXPRESS.

Statistical analysis

For the statistical analysis of our study, we used SPSS (Statistical Package for the Social Sciences, Chicago USA) Version 26.0 using which we generated the numeric relevant to our study.

Results

53 (35%) of the 150 total specimens were from ETT tips, whereas 97 (65%) came from tracheal aspirates. 101 (67%) of the organisms were gram-negative, and 49 (33%) were gram-positive. Acinetobacter spp. made up the bulk of the 101 gram-negative organisms, appearing in 53 (52%) of them, followed by Enterobacter spp. in 13 (13%) and Klebsiella spp. in 12. (12 percent). Streptococcus species were predominant in 16 (32%) of the 49 gram-positive samples, Methicillin-Resistant Staphylococcus aureus (MRSA) were discovered in 10 (21%) samples, Staphylococcus aureus were detected in 9 (18%) samples, and Staphylococcus epidermidis were found in 7 samples (15 percent).

Table 1, given below, is a tabular representation of the various bacterial species that we obtained through the endo tracheal aspirates and ETT tips of the patients

Type of bacterium	Specimen nomenclature	Percentage	Frequency (*n*)
Gram positive	*Methicillin-Resistant Staphylococcus aureus (MRSA)*	21%	10
	Staphylococcus aureus	18%	9
	Staphylococcus epidermidis	15%	7
	Streptococcus spp.	32%	16
	Acinetobacter baumannii	52%	53
Gram negative	*Burkholderia cepacia*	11%	11
	Escherichia coli	4%	4
	Enterobacter spp.	13%	13
	Klebsiella pneumoniae	12%	12
	Pseudomonas spp.	8%	8

Table 1: Tabular representation of the types of various bacterial species isolated from the samples obtained

Shown below in table 2 are the sources of our samples (in percentages) and the types of bacteria isolated through them on the basis of their gram staining.

Variable analysed	Variable characteristic	Frequency (*n*)	Percentage
Source of microbe	Endo trachael aspirate	97	65%
	ETT tips	53	35%
	Total	150	100%
Type of bacterium	Gram negative	101	67%
	Gram positive	49	33%
	Total	150	100%

Table 2: Tabular representation of the source of microbial species and the type of bacterium isolated in the study

Table 3, as can be seen below, shows the percentage of resistance to various antibiotics shown by the gram-negative bacterium identified in this study.

Antibiotic used	*Acinetobacter baumannii*	*Burkholderia cepacia*	*Escherichia coli*	*Enterobacter spp.*	*Klebsiella pneumoniae*	*Pseudomonas spp.*
Amikacin	68%	72%	71%	59%	65%	0%
Gentamicin	-	69%	82%	-	-	-
Doxycycline	9%	-	77%	-	-	-
Ciprofloxacin	-	-	62%	81%	77%	0%
Ceftriaxone	-	72%	-	89%	91%	-
Penicillin	-	54%	-	-	-	-
Piperacillin-tazobactam	93%	-	88%	36%	44%	3%
Vancomycin	-	-	-	-	-	-
Imipenem	-	-	-	31%	39%	0%

Oxacillin	-	-	-	-	-	-

Table 3: Percentage of resistance to various antibiotics shown by the gram-negative bacterium identified in this study

Table 4, as can be seen below, shows the percentage of resistance to various antibiotics shown by the gram-positive bacterium identified in this study.

Antibiotic used	*Methicillin-Resistant Staphylococcus aureus (MRSA)*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Streptococcus spp.*
Amikacin	-	-	-	-
Gentamicin	100%	98%	21%	-
Doxycycline	-	-	-	-
Ciprofloxacin	100%	63%	33%	-
Ceftriaxone	100%	0%	12%	48%
Penicillin	100%	27%	-	68%
Piperacillin-tazobactam	-	-	-	-
Vancomycin	0%	0%	0%	-
Imipenem	-	-	-	55%
Oxacillin	100%	0%	32%	-

Table 4: Percentage of resistance to various antibiotics shown by the gram-positive bacterium identified in this study

Figure 1 depicts the percentage of type of bacterium identified in our study on the basis of the findings mentioned in table 2.

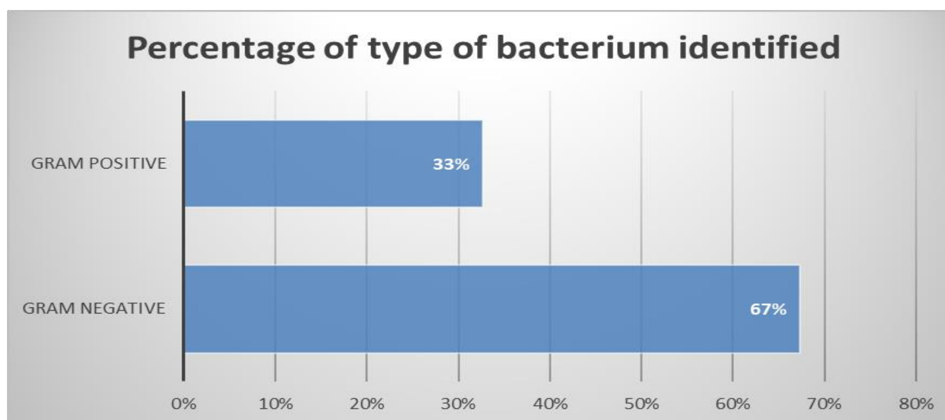

Figure 1: Percentage of type of bacterium identified in our study

Figure 2 depicts the percentage of source of microbial samples utilised in our study on the basis of the findings mentioned in table 2.

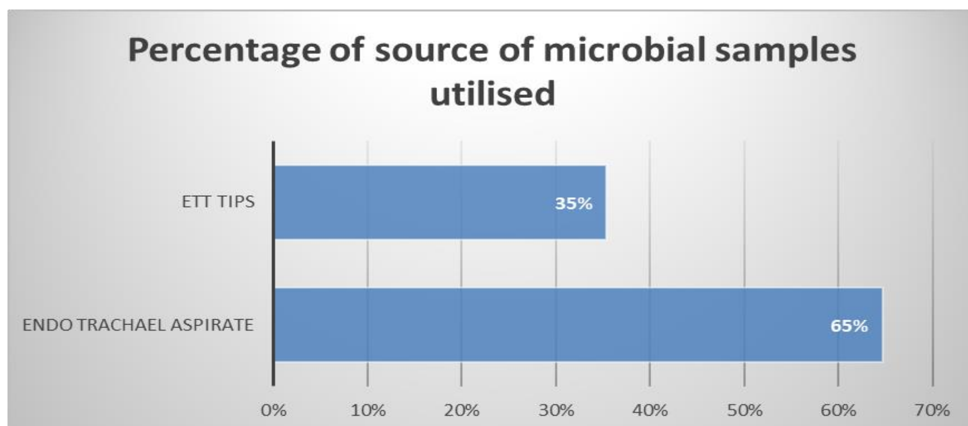

Figure 2: Percentage of source of microbial samples utilised in our study

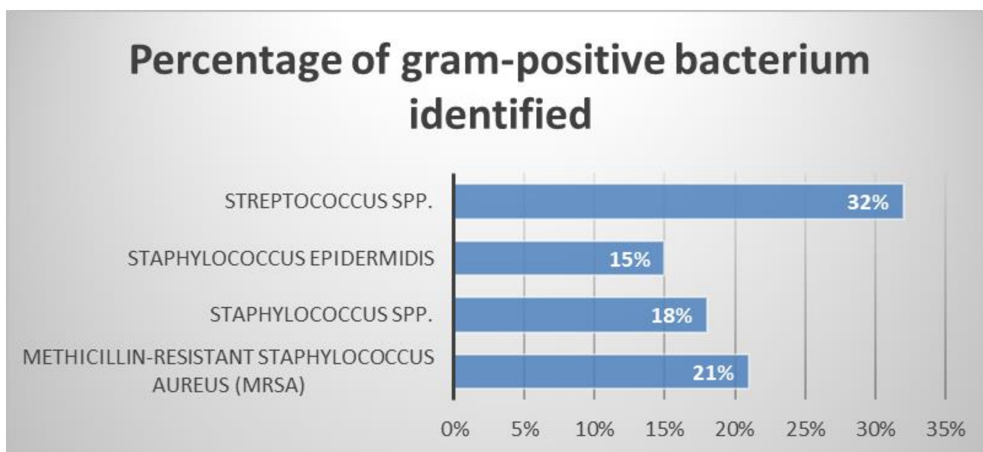

Figure 3 represents the percentage of gram-positive bacterium identified in our study

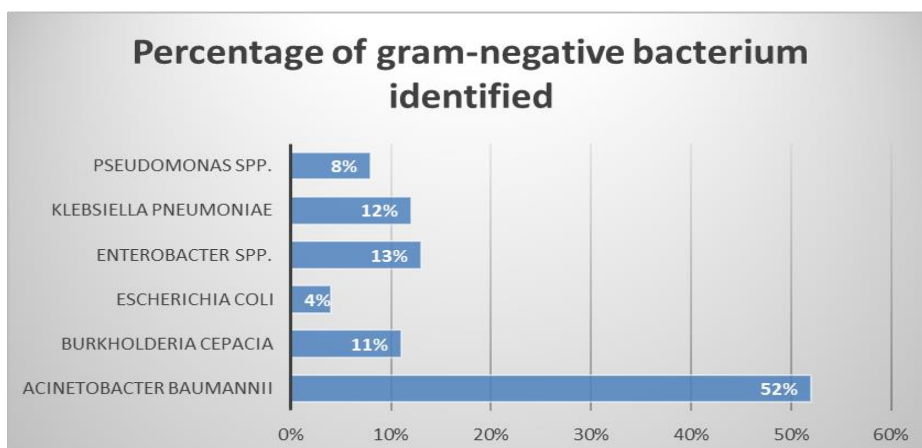

Figure 4: Percentage of gram-negative bacterium identified in our study

Figure 5 (a) and (b) display the group statistics and independent t-test results for the bacterium identified in this study, based upon our findings mentioned in table 1.

Group Statistics					
Variable	Bacterium	N	Mean	Std. Deviation	Std. Error Mean
Frequency	Gram-positive	5	9.8000	3.70135	1.65529
	Gram-negative	6	16.8333	18.01573	7.35489

Figure 5 (a): Group statistics for the gram-positive and gram-negative bacterium identified in our study

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference Lower	Upper
Frequency	Equal variances assumed	2.864	.125	-.851	9	.417	-7.03333	8.26729	-25.73523	11.66857
	Equal variances not assumed			-.933	5.502	.390	-7.03333	7.53886	-25.89293	11.82626

Figure 5 (b): Independent t-test results of the specimens identified in our study

Discussion

Lower respiratory tract infections are the most prevalent bacterial infections in ICU patients. Infectious Disease Society of America recently ranked the three types of bacteria that include carbapenem-resistant Acinetobacter spp., Echerichia coli and Klebsiella spp., and Multi-drug resistance (MDR) pseudomonas as the top bacterial pathogens. It was demonstrated that doxycycline was effective against Acinetobacter, and the resistance rate was 2.6%; however, we also noted a 4.9 percent resistance rate. [22] According to this study, Gram-negative bacteria were the most often isolated pathogens, while Gram-positive bacteria were more prevalent among patients who had been admitted to the intensive care unit. The most common strains were MRSA and Staphylococcus aureus. Testing for antibiotic susceptibility found that isolated bacteria in our setup were resistant to the majority of routinely used antibiotics.

The establishment of bacterial resistance presenting as a significant problem for the management and prevention of serious illnesses among patients is one of the main problems. [23] In order to improve clinical results, it is therefore essential to identify the local microbial flora and their antimicrobial susceptibility pattern. Similar to the findings of the present study, earlier investigations have noted that Klebsiella species were the most often isolated Gram-negative bacteria from

tracheal aspirates and ETT tips, followed by Acinetobacter, Pseudomonas, and Staphylococcus aureus. [24-25]

The results of the current investigation showed that while doxycycline was successful, Acinetobacter spp isolates were resistant to the majority of regularly used antibiotics. The outcomes are consistent with several published research [26], in which Acinetobacter was shown to be very susceptible to polymyxin-b and colistin, intermediately resistant to meropenem and imipenem, and highly resistant to piperacillin tazobactam. Kidwai et al. [27] also found very similar results, reporting 0% resistance to polymyxins, 9% amikacin resistance, and 69.4% piperacillin-tazobactam resistance. Similar findings, according to Panda et al, [28] showed that polymyxin-b, colistin, and meropenem all had 100% cure rates, but amikacin had a cure rate of 28.57 percent.

Similar results were found by Ranjan et al, [29] including resistance to amikacin 53.3%, ciprofloxacin 66.63%, meropenem 20.3%, piperacillin and tazobactam 13.3%, and ceftazidime 93.33%. In their review on the antimicrobial resistance of pathogens isolated from tracheal and endotracheal aspirates, Juayang et al. [30] reported that Pseudomonas had the highest resistance to ceftazidime (65.7%), ciprofloxacin (40.6%), aztreonam (33.3%), meropenem and imipenem (25%) and piperacillin-tazobactam (26.3%).

While another study [31] found that Pseudomonas was highly resistant to all antibiotics except for colistin, which had 0% resistance. In one of the investigations, [32] it was found that Klebsiella was susceptible to amikacin to the extent of 27.7%, ceftriaxone to the extent of 9.9%, meropenem to the extent of 90.9%, polymyxin-b to the extent of 100 percent, and colistin, while Pseudomonas was susceptible to amikacin to the extent of 60%, ceftazidime to the extent of 20%, meropenem to. Gram-positive Staphylococcus aureus in our study displayed 100% gentamicin resistance, 66.7 % MRSA resistance pattern as 100% resistance to all employed antibiotics, with the exception of vancomycin, for which both Gram-positive bacteria displayed 0% resistance. The outcomes are consistent with other published studies that claim there is zero vancomycin resistance. According to a study, compared to MRSA, methicillin-sensitive Staphylococcus aureus (MSSA) has a 50% resistance to penicillin and a 0% resistance to oxacillin, ciprofloxacin, gentamicin, and vancomycin. Penicillin and oxacillin revealed 100% resistance from the MRSA, while vancomycin, ciprofloxacin, and gentamicin showed 0% resistance.

Conclusion

As a result of bacterial colonisation, patients admitted to the ICU are substantially more likely to develop respiratory tract infections. Multi-drug resistance bacteria are brought on by the improper use of broad-spectrum antibiotics. Antibiotic resistance among these bacteria must be prevented immediately, and in the future, suitable empirical antibiotic therapy may be beneficial.

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