

COLONIZATION AND ANTIBIOTIC RESISTANCE DYNAMICS OF PATIENTS AT INTENSIVE CARE UNIT: OUR EXPERIENCE

ISHWAR CHANDRA BEHERA¹, SANTOSH KUMAR SWAIN², MAHESH CHANDRA SAHU^{3*}

¹Department of Community Medicine, IMS and SUM Hospital, Siksha "O" Anusandhan University, Bhubaneswar, Odisha, India.

²Department of Otorhinolaryngology, IMS and SUM Hospital, Siksha "O" Anusandhan University, Bhubaneswar, Odisha, India. ³Directorate of Medical Research, IMS and SUM Hospital, Siksha "O" Anusandhan University, Bhubaneswar, Odisha, India. Email: mchsahu@gmail.com

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ABSTRACT

Objective: Multidrug-resistant strains are developed day by day due to empirical therapy in intensive care unit (ICU). Both Gram-positive and Gram-negative strains are found in ICU are drug resistant bacteria. In this study, the prevalence of drug resistance bacteria in ICU were determined.

Methods: The clinical samples, ascitic fluid, blood, central line tip, ET aspiration pleural fluid, pus from liver abscess, sputum, tracheal aspiration, urine, and wound swabs were collected from ICU patients and cultured in specific medium. Subsequently, antibiotic sensitivity pattern of isolated bacteria was carried out with disc diffusion methods.

Results: A total number of 794 clinical samples were cultured in specific medium, and it revealed 191 of single colonies, 145 were double colonies and 87 were 3 or Multy-colonies. A total of 963 bacteria were isolated among them 180 were Gram-positive bacteria, budding yeast-like colony were 135 and rest were Gram-negative bacteria. There was no significant difference in growth of microorganisms with respect to clinical samples as t-test revealed $p=0.87$. Among the Gram-negative bacteria; *Klebsiella pneumonia* were predominant, and *Proteous mirabilis* was lowest. Tigecycline and colistin were most effective antibiotics agonist all bacteria.

Conclusion: Urine samples of the patient's revealed a higher number of organisms whereas pus from liver abscess found only one number of growths. The antibiotic stewardship programs must lead to significant decrease in all antibiotic used in the ICU setting. However, this result may be required further work to determine the fruitful necessary for success.

Keywords: Antibiotics, Intensive care unit, Bacterial infections, Drug resistance.

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INTRODUCTION

Antibiotic resistance is the most severe threats to public health today [1]. It is well documented that misuse of antibiotics contributes to develop of multidrug resistance strains and studies have shown that up to 50% of antibiotics use in clinical practice are inappropriate [2,3]. Antibiotic stewardship programs (ASPs) are highly being advised as key strategy to increase the suitability of antibiotic prescribing, with the aim of avoiding the emergence of resistance [1,3]. Some more advantage of appropriate antibiotic use results the reduction in adverse, including intensive care unit (ICU) infection and drug reactions, as well as a decrease in health-care costs [4,5]. The World Health Organization (WHO), the Centers for Disease Control and Prevention along with the Infectious Diseases Society of America all endorse ASPs as an effective sources to prevent the increase and spread of antibiotic resistance [1,6,7]. One of the most promising ASP intervention strategies is to decrease the drug resistant strains and provide the accurate antibiotics to the ICU patients [8,9]. There are several studies evaluate the prospective audit and feedback programs have been conducted on wards, cabins and ICUs may be the setting with the greatest potential impact [8-10]. All ICU patients are prescribed empirical antibiotics which revealed the high levels of antibiotic resistance [10,11]. On the other hand, inadequate initial antibiotic therapy has been associated with mortality in critically ill patients [12]. Still, now, there have been some studies which provide the impact of audit and feedback in ICUs [11,13-16].

ICU patients are mostly immunocompromised, and they need specific drug during their treatments. ICUs are one of the sources for the containment of antibiotic resistance. Nosocomial infections are the major cause for cross contamination to seriously ill patients, and it is

due to the indiscriminate use of combined antibiotics and has resulted in highly resistant bacteria pathogens [17]. Infections caused by drug-resistant microorganisms have higher morbidity and mortality rates and higher costs as compare to antibiotic sensitive bacterial infections [18-20]. The prevalence of combination antibiotic resistance in microorganism is higher among isolates from patients admitted to ICU than that among non-ICU inpatients [21-23]. Gram-negative bacteria including *Pseudomonas aeruginosa* have emerged as key reason for nosocomial infections and account for around 30% and 5%, respectively, of all bloodstream infections [24,25]. Antibiotic resistance surveillance programs have verified an increase in resistance among these Gram-negative bacteria [23-26]. Bacterial colonization is often a primary step in the pathogenesis of nosocomial infections. Therefore, the choice of empirical antibiotic therapy depends, at least partly, on the colonization and resistance of the microorganisms. Verities of mechanisms may lead to the colonization of hospitalized patients with resistant strains [27,28]. The patients, after discharging from the hospital can spread the drug-resistant bacteria in the community. These resistant strains survive for a long period and performed to develop infections with resistant bacteria [29,30]. These studies will be limited value for major changes in antibiotic resistance of the microorganisms. Few other studies revealed that there were no any drug-resistant bacteria after discharge from the hospital [31-33]. Many studies did not identify the microorganisms for species label [30-32,34]. According to these studies, an observed increase in resistance in Gram-negative bacteria may, in fact, have been the result for verities of species of the organisms [33]. In this study, we isolate the organism from different clinical samples from the ICU patients and identifies with species label. After identification, the percentage of sensitive and resistance of antibiotics with concerned microorganisms were determined.

METHODS

In this prospective study and approve from the Institutional Ethics Committee. The clinical samples; ascitic fluid, blood, central line tip, pleural fluid, pus from liver abscess, sputum, tracheal aspiration, urine and wound swabs were collected from the patients of neuro-ICU. All the clinical samples were cultured in suitable culture media and incubated at 37°C for 18 hrs. For pure culture, individually colonies were streaked in an agar plate and then processed (Fig. 1). The bacterial colony morphology was noted (Table 1) for further identifications with Gram stain and biochemical tests.

All Gram-negative bacteria were identified basing on the biochemical results (Table 2), whereas all Gram-negative bacteria were identified basing upon the test, catalase, oxidase, and coagulase results. The results are compared with the colony morphology of the culture result also. In *Staphylococcus aureus* golden yellow, opaque, circular colonies white butyrous consistency were observed on nutrient agar

whereas, yellow colonies were observed on mannitol salt agar, and beta-hemolysis was seen on blood agar. After identification, individual bacterial were tested for antibiotic sensitivity pattern with Kirby-Bauer method. Then, the sensitive or resistances of the used antibiotics were detected by measuring the diameter of inhibitor zone created by the antibiotics (Fig. 2). All the organisms were identified basing on the previous methods [18-25].

RESULTS

A total number of 794 clinical samples from the neuro-ICU patients were cultured on suitable media. It is revealed that among them 371 did not grow on the microbiological culture media. Whereas rest samples grew in the culture media and showed single colony, double colony, and 3 or more colonies (Table 3).

There was a t-test compared with the number growth samples and number of no growth samples, and it was revealed that there was

Table 1: Morphology and culture characters of clinically isolated Gram-negative bacteria along with MTCC strains

Bacterium	Agar media	Colony morphology
<i>Acinetobacter baumannii</i>	Nutrient agar (NA)	Colorless smooth raised and pinpoint colonies
	MacConkey (MAC) agar	Colorless smooth, raised, NLF colonies
<i>Enterobacter aerogenes</i>	BA	Small, round and pin-point colony
	MAC agar	LF and mucoid colonies
<i>Escherichia coli</i>	NA	Flat dry, irregular colonies
	MAC agar	LF, flat dry pink, irregular colonies
	EMB agar	Flat dry, irregular colonies, with metallic green color
	CLED agar	translucent blue colonies
<i>Klebsiella pneumoniae</i>	MAC agar	LF, pink, mucoid colonies
	CLED agar	Yellow and mucoid colonies
<i>Proteus vulgaris</i>	BA	Swarming colonies
	CLED agar	translucent blue colonies
<i>Pseudomonas aeruginosa</i>	NA	Large, irregular colonies, with bluish green pigment
	MAC agar	Pale and yellowish (NLF), translucent without black center
	XLD agar	Red without black center

NA: Not available, EMB: Eosin methylene blue agar, XLD: Xylose lysine deoxycholate, CLED: Cystine lactose electrolyte deficient medium, LF: Lactose fermenting, NLF: Non-lactose fermenting, BA: Blood agar

Table 2: Summary of results of biochemical tests of isolated Gram-negative bacteria

Bacteria	Catalase	Oxidase	Indole	MR	VP	Citrate	Urease	TSI	Nitrate	M
<i>A. baumannii</i>	+	ND	-	-	+	+	V	ND	-	M
<i>Enterobacter aerogenes</i>	+	ND	-	-	+	+	V	A/A	+	M
<i>Escherichia coli</i>	+	ND	+	+	-	-	-	A/AG	+	M
<i>Klebsiella oxytoca</i>	+	ND	+	-	+	+	+	A/AG	+	NM
<i>Klebsiella pneumoniae</i>	+	ND	-	-	+	+	+	A/AG	+	NM
<i>Proteus vulgaris</i>	+	ND	-	+	-	V	+	K/A H ₂ S	+	M
<i>Proteus mirabilis</i>	+	ND	+	+	-	V	+	K/A H ₂ S	+	M
<i>Pseudomonas aeruginosa</i>	+	+	-	-	-	+	+	ND	+	M

+: Positive, -: Negative, V: Variable, MR: Methyl red, VP: Voges-Proskauer, TSI: Triple sugar iron, A: Acid, K: Alkali, G: Gas, H₂S: H₂S production, M: Motile, NM: Non-motile, ND: Not done

Table 3: Types of colonies from different clinical samples of patients

Sample	No growth	Single colony	Double colony	3 or more colony	Total growth	Total samples
Ascitic fluid	14	2	0	0	2	16
Blood	122	88	32	34	154	276
Central line tip	1	4	0	0	4	5
ET aspiration	2	2	0	0	2	4
Pleural fluid	0	2	0	0	2	2
Pus from liver abscess	7	1	0	0	1	8
Sputum	5	7	3	9	19	24
Tracheal aspiration	4	2	2	10	14	18
Urine	198	79	105	22	206	404
Wound swab	18	4	3	12	19	37
Mean±SD	37.10±67.44	19.10±34.05	14.50±33.28	8.70±11.59	42.30±73.94	79.40±141.02
Total	371	191	145	87		794

SD: Standard deviation

Table 4: Frequency of isolated bacteria from different samples of the patients admitted at ICU.

Sample/organism	Ascitic fluid	Blood	Central line tip	ET aspiration	Pleural fluid	Pus from liver abscess	Sputum	Tracheal aspiration	Urine	Wound swab	Total
<i>Staphylococcus aureus</i>	10	28	14	2	0	21	22	11	26	46	180
<i>Acinetobacter</i> Sp.	1	42	5	3	0	0	12	4	13	2	82
<i>Escherichia coli</i>	9	2	0	0	0	0	6	0	43	3	63
<i>Enterobacter</i> Sp.	0	0	1	0	0	0	2	0	5	1	9
<i>Enterococcus</i> Spp.	0	2	0	0	0	2	4	0	0	2	10
<i>Klebsiella pneumoniae</i>	2	18	29	36	4	0	37	79	9	16	230
<i>Klebsiella oxytoca</i>	0	12	21	28	0	0	25	39	4	18	147
<i>Proteus mirabilis</i>	0	4	0	0	0	0	0	0	2	0	6
<i>Proteus vulgaris</i>	0	9	0	0	0	0	0	0	0	0	9
<i>Pseudomonas auregenosa</i>	2	6	3	0	0	0	9	2	28	42	92
BYC	0	27	3	1	0	0	1	0	94	9	135
Mean±SD	2.18±3.71	13.64±13.57	6.91±9.98	6.36±12.84	0.36±1.21	2.09±6.30	10.73±12.22	12.27±24.99	20.36±28.07	12.64±16.74	87.55±77.64
p value	0.04*	0.48	0.14	0.14	0.02*	0.04*	0.30	0.48	1.00	0.44	0.01*
Total	24	150	76	70	4	23	118	135	224	139	963

BYC: Budding yeast-like colony. *represents the significant of the study with t-test, with a comparison of a number of bacteria isolated from urine to other clinical samples. SD: Standard deviation



Fig. 1: Isolation of single colony with streak plate method

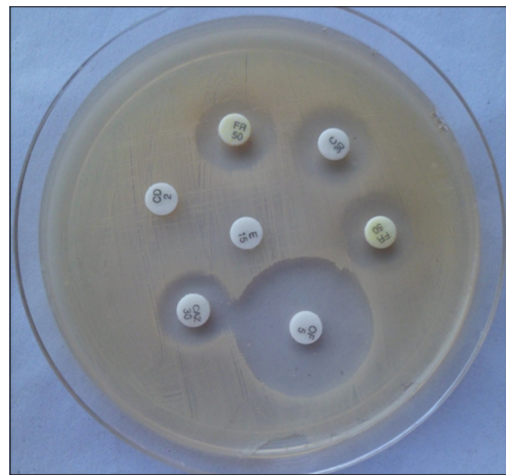


Fig. 2: Antibiotic sensitivity pattern of bacteria by disc diffusion methods

no any statistically significantly as $p=0.87$. Thus, the samples of the ICU patients may or may not contain microorganisms for growth as it is equally distributed according to t-test. All colonies were tested biochemically for the identification of bacteria. It is found that *Klebsiella pneumoniae* (230) is the major contaminated bacteria in neuro-ICU. Minimum contaminated, i.e., 9 *Proteus vulgaris* were isolated from the clinical samples of the neuro-ICU patients (Table 4). The organism isolated with different clinical samples were compared with t-test with urine sample and revealed significant with abscess fluid ($p=0.04$), pleural fluid (0.02), pus from liver abscess ($p=0.04$), and with total sample ($p=0.01$) (Tables 5 and 6).

DISCUSSION

This study documents the microorganisms isolated from ICU and subsequently found the sensitivity and resistance of antibiotics with concerned microorganisms. The prevalence of Gram-negative bacteria is found more as compared to the Gram-positive bacteria in ICU. Sometimes, patients under treatment with antibiotics revealed suppression of growth in culture medium [35]. Hospital practice may be the cause for these cases. This hypothesis is supported by the fact that the growth of bacteria rates at different times and between the two populations were inversely related to use of antibiotic at the measured levels. Thus, the higher the level of antibiotics used, the lower the bacterial colonization rate. Second, either fresh urine specimens or some other samples were collected, and a bias may have been introduced at this point [36]. Other studies have also reported

Table 5: Percentage of antibiotic resistance to *Staphylococcus aureus*

Bacteria	Susceptibility to prescribed antibiotics															
	AK	Ge	Ac	Am	Ox	P	Ctr	Cf	Of	Tei	Va	E	Az	Cd	TGC	COL
<i>Staphylococcus aureus</i>	75	46	43	38	87	59	85	68	53	48	27	43	62	39	05	09

Antibiotics ($\mu\text{g}/\text{disc}$): Ak: Amikacin 30, Ac: Amoxycylav 30, Am: Ampicillin 10, Cf: Cefpodoxime 10, Ctr: Ceftriaxone 30, Ge: Gentamicin 30, Of: Ofloxacin 5, Ox: Oxacillin 1, P: Penicillin 10, Az: Azithromycin 15, Cd: Clindamycin 2, Ch: Chloramphenicol 30, Cot: Co-trimoxazole 25, E: Erythromycin 15, Tei: Teicoplanin 30, Va: Vancomycin 30

Table 6: Percentage of antibiotic resistance to Gram-negative bacteria

Bacteria	Susceptibility to prescribed antibiotics															
	AK	GEN	NET	TOB	AT	PI	PIT	CPM	CPZ	CFS	CAZ	CTR	IPM	GAT	TGC	COL
<i>A. baumannii</i>	23	30	20	14	71	30	21	71	62	34	78	72	21	20	2	9
<i>Enterobacter aerogenes</i>	7	8	12	31	63	40	39	69	52	15	82	74	31	22	7	14
<i>Escherichia coli</i>	78	56	51	32	60	78	34	61	56	24	74	68	42	16	15	22
<i>Klebsiella oxytoca</i>	24	36	32	16	74	82	25	54	62	21	82	74	38	19	18	19
<i>Klebsiella pneumoniae</i>	63	83	62	21	61	87	28	57	71	21	89	82	56	71	18	17
<i>Proteus vulgaris</i>	69	48	81	25	58	91	34	64	52	16	91	92	58	69	24	26
<i>Proteus mirabilis</i>	49	54	31	23	46	69	21	78	61	14	78	79	37	78	26	27
<i>Pseudomonas aeruginosa</i>	68	59	28	17	51	67	23	83	73	31	73	74	39	31	11	17

Antibiotics ($\mu\text{g}/\text{disc}$): AK: Amikacin 30, GEN: Gentamicin 30, NET: Netillin 30, TOB: Tobramycin 10, AT: Aztreonam 30, PI: Piperacillin 100, PIT: Piperacillin/tazobactam 100/10, CPM: Cefepime 30, CPZ: Cefoperazone 75, CFS: Cefoperazone/sulbactam 75/30, CAZ: Ceftazidime 10, CTR: Ceftriaxone 10, IPM: Imipenem 10 (carbam, carbapenem), GAT: Gatifloxacin 30, TGC: Tigecycline 55; COL: Colistin 10

low colonization rates of Gram-negative or Gram-positive bacteria in hospitalized patients [37-39].

In a study, daily use of antibiotics was reduced 28% in the TNICU (1433 vs. 1037) but increased 14% in the MSICU (1705 vs. 1936). The total monthly antimicrobial use in the TNICU decreased by 375 DDD per 1000 patient days ($p < 0.0009$) immediately following the intervention, followed by a nonsignificant downward trend in use of - 9 DDD per 1000 patient days ($p = 0.56$) [40].

A high prevalence of health care-associated infections was observed, mainly caused by Gram-negative bacteria with high carbapenem resistance rates. This in combination with a high rate of antimicrobial use illustrates the urgent need to improve rational antimicrobial use and infection control efforts [41-43]. However, from a study, it was partially confirmed that ICU patients would be exposed to water amoeba-associated microorganisms and provides information about the magnitude of AAM infection in ICU patients, especially patients that have a prolonged ICU stay. However, the incidence of this exposure on the development of pneumonia remains to assess [44]. The use of antibiotics and its resistance became significant challenges for patient safety as well as management of the critically ill on ICU. ASPs aim to optimize appropriate antibiotic treatment while minimizing antibiotic resistance. Different models of ASP in ICU, include "standard" control of antibiotic prescribing such as "de-escalation strategies" through to interventional approaches utilizing biomarker-guided antibiotic prescribing. No study has demonstrated a survival benefit of ASP. Ongoing challenges to infectious disease management, reported by the WHO global report 2014, are high AMR to newer antibiotics, and regional knowledge gaps in AMR surveillance. Improved AMR surveillance data, identifying core aspects of successful ASPs that are transferable and further well-conducted trials will be necessary if ASPs are to be an effective platform for delivering desired patient outcomes and Safety through best antibiotic policy [45].

CONCLUSIONS

This study resulted the most common sources of infection on admission to the ICU. The length of hospital stay in patients with infections is longer, likely because of the increased numbers of secondary infections in these patients. However, mortality rates were identical in these groups of patients. Our results demonstrate the potential for audit and feedback to significantly reduce antimicrobial use in ICU settings.

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