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Antibiogram Analysis of Gram Negative Bacterial Isolates from Various Clinical Specimens in Puducherry, South India.

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ABSTRACT

This study was performed in two groups as inpatient (IP) samples and outpatient (OP) samples in Government general Hospital, Puducherry. The present study was carried out to determine the antibiotic susceptibility pattern of gram negative isolates from various clinical specimens like urine, pus, stool, blood and other bodily fluids. Totally 412 samples were collected from suspected patients. Out of 412 samples, 322 (78.2%) showed gram negative bacterial isolates in which 101 (31.4%) outpatients' and 221 (68.6%) inpatients' samples. Various bacterial isolates like *Escherichia coli* (42.24%), *Klebsiella sp.*, (26.21 %), *Pseudomonas sp.*, (13.75%), *Salmonella sp.*, (10.25%), *Proteus sp.*, (4.66%), *Acinetobacter sp.*, (1.55%), *Aeromonas sp.*, (0.31%), *Enterobacter sp.*, (0.31%), and *Gardnerella vaginalis* (0.62%) were isolated and identified up to generic level based on the colony characteristics, gram staining and biochemical characteristics as. Susceptibility pattern of gram negative isolates showed that Cefoperazone/sulbactam was the most effective antibiotic irrespective of clinical specimens.

Keywords: Antibiogram, Gram Negative Bacteria, Antibiotics, Antibiotic Susceptibility, Antibiotic Resistance, Cefoperazone/sulbactam.

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INTRODUCTION

The mechanism of action of antibiotics and its usefulness in clinical industry has long been known but the life threatening microbes are also becoming resistant to most available antibiotics [1,2]. India said to be highest burden countries for bacterial infections in world and the usage of antibiotics play substantial role in morbidity and mortality [3]. Modernization and alteration of natural habitation humans are more prone to acquire infection especially in hospital acquired infections which is considerably life threatening to patient and health care professionals in hospital [4-6]. Gram negative organisms are major more common causative agents of variety of infections and are closely associated with patient's age [2].

Administration of repetitive antibiotic usage, improper and higher dose results in the development of multidrug resistant strains which is very commonly associated with gram negative bacteria [4,7]. More importantly arising of drug resistance among gram negative bacilli is of clinical importance and pose serious threat to public health. Numerous studies were performed to identify susceptibility patterns of gram negative bacterial isolates. Thus, this study was performed in Pus, Blood, Urine, Stool, Sputum and other samples collected from inpatient (IP) and outpatient (OP) in Government general Hospital. The samples were subjected to identify gram negative bacteria like *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi*, *Acinetobacter sp.*, *Aeromonas sp.*, *Enterobacter sp.*, and *Gardnerella vaginalis*. Therefore, it was attempted to explore the distribution and antibiotic susceptibility pattern of gram negative isolates of clinical importance among the clinical specimens analyzed for routine laboratory diagnostics.

MATERIALS AND METHODS

Collection of Samples

In this retrospective study, Totally 412 samples were collected from various clinical specimens of patients attended and hospitalized between in Government general Hospital, Puducherry. Pus, Blood, Urine, Stool, Sputum and other samples were collected from in-patients and out-patients following the method as described earlier [8]. Samples were inoculated in appropriate media for isolation as listed in table 1.

Processing of Specimens [8,10,11]

All the samples were inoculated with suitable medium and incubated aerobically at 37°C for 24 - 48 hrs. Plates showing colonies were selected and gram staining was performed to identify the organism. The strains were identified by standard bacteriological methods, using the criteria based on the Bergey's manual of systematic bacteriology. The characteristics of organisms used for identification include colony morphology, gram staining and biochemical tests (indole test, methyl red test, voges proskauer test, citrate utilization test, urease test, triple sugar iron agar test (TSI), catalase test and oxidase test).

Antibiotic Susceptibility Test

Antibiotic susceptibility pattern of each isolated bacterial strain to various antibiotic was performed by Kirby-Bauer disc diffusion method as described [12].

The Following Antibiotics Were Used

Amikacin (30mcg/disc), Ampicillin (10mcg/disc), Carbenicillin (100mcg/disc), Cefoperazone/sulbactam (75mcg/disc), Ceftazidime (30mcg/disc), Ceftriaxone (30mcg/disc), Cephalexin (30mcg/disc), Cephodoxime (30mcg/disc), Chloramphenicol (30mcg/disc), Ciprofloxacin (5mcg/disc), Gentamicin(10mcg/disc), Nalidixic Acid (30mcg/disc), Nitrofurantoin (300mcg/disc), Norfloxacin (10mcg/disc), Ofloxacin (5mcg/disc), Sparfloxacin (5 mcg/disc). All the antibiotic discs used in this study were purchased from HiMedia, India.

Measuring of Zone of Inhibition

After 24 hrs of incubation the diameter of zone of inhibition was measured in millimeter (mm) and the sensitivity pattern of each isolate was recorded. The values were interpreted by standard

references, as Sensitive (S), Intermediate (I) and Resistant (R). The results of the susceptibility testing were classified into two categories. The category "susceptible" was defined as identification of a strain as susceptible by the disc diffusion method or micro dilution technique. All resistant and intermediate isolates of the species were classified under the definition "resistant" [13].

Zone of inhibition results against each antibiotic disc was measured based on the inhibition zone size scale provided by the manufacturer (HiMedia, India).

RESULTS AND DISCUSSION

A total of 412 clinical samples were analyzed for the presence of gram negative bacterial pathogens. Ninety (21.8%) samples were found as normal/gram positive microbial flora and 322 (78.2%) samples were analyzed for further procedures. In which, *E. coli* was constituted as major pathogens and its frequency was about 42.24%. The other bacterial isolates frequency is listed in Table 2. Table 2 also shows the nature of clinical sample and the distribution of gram negative organisms isolated in clinical specimens. Out of 322 samples, inpatients samples were 221 (68.63%) and outpatient samples were 101 (31.37%). Out of 322 samples were included in this study male was 162 (50.31%) and female was 160 (49.69%). Table 3 summarizes frequency and distribution of gram negative organisms isolated from in- and out-patients included in this study. Totally seven types of antibiotics group was used in this study which constitutes, Aminopenicillins, Quinolones, Fluoroquinolones, Aminoglycosides, Cephalosporins, Tetracycline and Miscellaneous. Figure 1 and Figure 2 represent an example of the susceptibility pattern and resistant for each antibiotic tested in this study.

Figure 1: Examples of antibiotic susceptibility pattern of gram negative isolates. Gram-negative isolates showed susceptibility to all the antibiotics used in this study.



Figure 2: Examples of antibiotic resistant pattern of gram negative isolates. Gram-negative isolates showed resistant to other antibiotics and showed susceptibility to cefoperazone/sulbactam antibiotic.

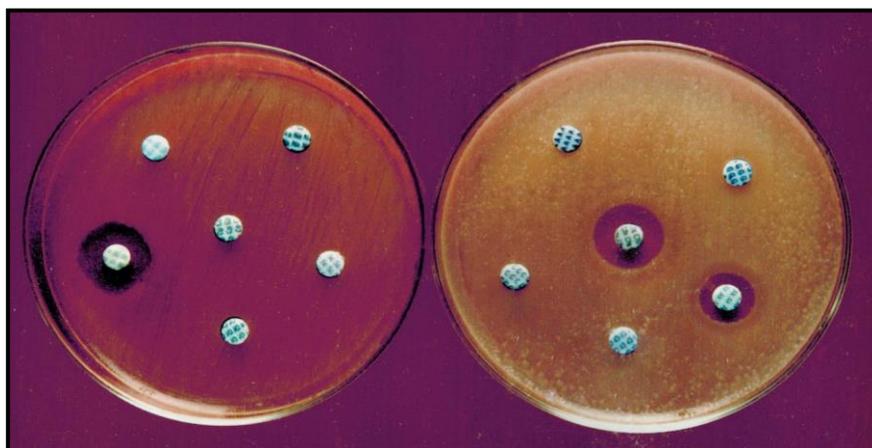


Table 1: Choices of Media used in this study [9].

S. No	Clinical specimen/test	Choice of media used
1.	Urine	MacConkey Agar and Blood Agar
2.	Rs	MacConkey Agar, Mannitol Salt Agar, Fluid Thioglycollate Medium, Blood Agar
3.	Sputum	MacConkey Agar, Blood Agar and Chocolate Agar.
4.	Stool	<i>Salmonella Shigella</i> Agar, MacConkey Agar, Selenite F broth, Hektone Enteric Agar
5.	Throat swab & other swabs	Blood Agar, MacConkey Agar, Chocolate Agar
6.	Blood Culture	For Adult-10 ml Blood into 50 ml Brain Heart Infusion (BM) broth. For Children-2 ml Blood into 25ml Brain Heart Infusion (Bill) broth
7.	Susceptibility test	Mueller Hinton Agar

All the media used in this study were purchased from HiMedia, India.

Table 2: Distribution of Gram Negative Bacterial Isolates

S. No.	Samples	No. of Isolates (%)	Organisms								
			<i>E.coli</i>	<i>Klebsiella sp.,</i>	<i>Pseudomonas sp.,</i>	<i>Proteus sp.,</i>	<i>Salmonella sp.,</i>	<i>Acinetobacter sp.,</i>	<i>Aeromonas sp.,</i>	<i>Gardnerella vaginalis</i>	<i>Enterobacter sp.,</i>
1	Urine	138 (42.86)	94	26	11	4	-	3	-	-	-
2	Pus	76 (23.60)	17	63	25	9	-	1	-	-	1
3	Stool	14 (4.35)	10	-	-	-	4	-	-	-	-
4	Blood	49 (15.22)	5	9	2	2	29	1	1	-	-
5	Sputum	20 (6.21)	3	17	-	-	-	-	-	-	-
6	Throat swab	11 (3.42)	-	8	3	-	-	-	-	-	-
7	Vaginal swab	5 (1.55)	2	1	-	-	-	-	-	2	-
8	Catheter tip	4 (1.24)	-	2	2	-	-	-	-	-	-
9	Semen	5 (1.55)	5	-	-	-	-	-	-	-	-
	Total	322 (100)	136(42.24%)	86 (26.71%)	43 (13.35%)	15 (4.66%)	33 (10.25%)	5 (1.55%)	1 (0.31%)	2 (0.62%)	1 (0.31%)



Table 3: Frequency of Gram Negative pathogens in Outpatients and Inpatients samples

S. No.	organism & No. of Isolates	Frequency of gram negative pathogens	
		OP	IP
1	<i>E.coli</i> (136)	48 (35.29%)	88 (64.71%)
2	<i>Klebsiella sp.</i> , (86)	24 (27.91%)	62 (72.09%)
3	<i>Pseudomonas sp.</i> , (43)	13 (30.23%)	30 (69.77%)
4	<i>Proteus sp.</i> , (15)	6 (40%)	9 (60%)
5	<i>Salmonella sp.</i> , (33)	6 (18.18%)	27 (81.82%)
6	<i>Acinetobacter sp.</i> ,(5)	2 (40%)	3 (60%)
7	<i>Aeromonas sp.</i> , (1)	-	1 (100%)
8	<i>Gardnerella vaginalis</i> (2)	2 (100%)	-
9	<i>Enterobacter sp.</i> ,(1)	-	1 (100%)
Total		101	221



Table 4: Susceptibility Patterns of Gram Negative Isolates

Samples and organism	ANTIBIOTICS																	
	N	%	G	C	CP	CI	CF	NA	NX	CFS	A	CA	CE	AK	CB	SC	OF	NF
Urine (138)																		
<i>E. coli</i>	94	68.12	51.06	67.02	44.68	38.30	46.81	54.26	39.36	62.77	40.43	46.81	48.94	53.19	34.04	39.36	31.91	41.49
<i>Klebsiella sp.,</i>	26	18.84	46.15	38.46	19.23	34.26	53.85	53.85	30.77	76.92	34.62	26.92	52.85	34.62	38.46	50.00	46.15	57.76
<i>Pseudomonas sp.,</i>	11	7.97	27.27	9.09	9.09	18.18	45.45	27.27	36.36	72.72	36.36	45.45	54.55	54.55	36.36	18.18	45.45	27.27
<i>Proteus sp.,</i>	4	2.30	25	25	100	0	75	50	50	75	50	25	75	50	25	50	75	75
<i>Acinetobacter sp.,</i>	3	2.17	100	66.67	100	33.33	100	33.33	66.67	66.67	66.67	66.67	66.67	33.33	66.67	33.33	66.67	100
Pus (76)																		
<i>E. coli</i>	17	22.37	76.47	82.35	64.71	41.18	64.71	NA	NA	88.24	52.94	41.18	35.29	82.35	76.47	58.82	64.71	NA
<i>Klebsiella sp.,</i>	23	30.26	65.21	82.61	60.87	43.48	86.96	NA	NA	91.30	86.96	52.17	78.26	86.96	43.48	82.61	86.96	NA
<i>Pseudomonas sp.,</i>	25	32.90	60	76	40	36	76	NA	NA	88	40	60	76	72	40	68	64	NA
<i>Proteus sp.,</i>	9	11.84	33.33	55.56	33.33	44.44	77.78	NA	NA	88.89	55.56	44.44	66.67	66.67	55.56	44.44	77.78	NA
<i>Enterobacter sp.,</i>	1	1.32	0	0	0	0	0	NA	NA	100	0	0	0	0	0	0	0	NA
<i>Acinetobacter sp.,</i>	1	1.32	100	100	100	100	100	NA	NA	100	100	100	100	100	100	100	100	NA
Stool (14)																		
<i>E. coli</i>	10	71.43	80	80	70	80	70	NA	NA	100	40	50	80	40	40	40	60	NA
<i>Salmonella sp.,</i>	4	28.57	25	75	25	25	100	NA	NA	100	50	50	75	50	25	50	75	NA
Blood (49)																		
<i>Salmonella sp.,</i>	29	59.18	93.10	96.55	89.76	82.76	96.55	NA	NA	100	96.55	75.86	79.31	96.55	89.66	96.55	96.55	NA
<i>E. coli</i>	5	10.20	40	80	40	40	60	NA	NA	100	60	40	40	60	40	60	60	NA
<i>Klebsiella sp.,</i>	9	18.37	77.78	66.67	77.78	55.56	77.78	NA	NA	88.89	77.78	66.67	77.78	77.78	66.67	44.44	55.56	NA
<i>Pseudomonas sp.,</i>	2	4.08	50	50	50	50	100	NA	NA	50	100	50	50	50	50	50	50	NA
<i>Proteus sp.,</i>	2	4.08	100	100	100	100	100	NA	NA	100	100	100	100	100	100	100	100	NA
<i>Acinetobacter sp.,</i>	1	2.04	100	100	100	100	100	NA	NA	100	100	100	100	100	100	100	100	NA
<i>Aeromonas sp.,</i>	1	2.04	100	100	100	100	100	NA	NA	100	100	100	100	100	100	100	100	NA
Sputum (20)																		
<i>Klebsiella pneumoniae</i>	17	85.00	52.94	64.71	52.94	52.94	70.59	NA	NA	70.59	23.60	41.06	58.82	70.59	47.06	35.29	70.59	NA
<i>E. coli</i>	3	15.00	33.33	33.33	66.67	33.33	66.67	NA	NA	100	66.67	33.33	33.33	100	33.33	33.33	66.67	NA
Throat swab (11)																		
<i>Klebsiella pneumoniae</i>	8	72.73	62.5	87.5	50	50	75	NA	NA	87.5	12.5	12.5	50	75	50	12.5	75	NA
<i>Pseudomonas sp.,</i>	3	27.27	66.67	100	33.33	66.67	100	NA	NA	100	33.33	33.33	66.67	100	66.67	66.67	66.67	NA
Vaginal swab(5)																		
<i>E. coli</i>	2	40	50	100	100	100	100	NA	NA	100	50	50	100	50	0	50	0	NA
<i>Klebsiella sp.,</i>	1	20	100	0	0	0	100	NA	NA	100	0	0	0	100	0	100	0	NA
<i>Gardnerella vaginalis</i>	2	40	50	100	50	50	100	NA	NA	50	100	50	100	100	50	50	50	NA
Seman (5)																		
<i>E. coli</i>	5	100	100	60	60	40	40	NA	NA	100	20	20	20	60	60	80	80	NA
Catheter tip (4)																		
<i>Klebsiella sp.,</i>	2	50	0	100	0	0	50	100	100	50	0	0	0	0	50	0	0	50
<i>Pseudomonas sp.,</i>	2	50	50	100	50	100	100	100	100	50	50	50	100	100	50	50	50	100

Note: NA - Note applicable, N-Number of Isolates, %- Percentage of occurrence, G - Gentamicin, C - Chloramphenicol, CP - Cephalexin, CI - Ceftriaxone, CF - Ciprofloxacin, NA - Nalidixic Acid, NX - Norfloxacin, CFS - Cefoperazone/Sulbactam, A - Ampicillin, CA -Ceftazidime, CE - Cephotaxime, Ak - Amikacin, CB - Carbenicillin, SC - Sparfloxacin, OF - Ofloxacin, NF – Nitrofurantoin.

Table 5: Resistant Patterns of Gram Negative Isolates

Samples and organism	ANTIBIOTICS																	
	N	%	G	C	CP	CI	CF	NA	NX	CFS	A	CA	CE	AK	CB	SC	OF	NF
Urine (138)																		
<i>E. coli</i>	94	68.12	48.94	32.98	55.32	61.7	53.19	45.74	60.64	37.23	59.57	53.19	51.06	46.81	65.96	60.64	68.09	58.51
<i>Klebsiella sp.,</i>	26	18.84	5.85	61.54	80.77	65.62	46.15	46.15	69.23	23.08	65.38	73.08	46.15	65.38	61.54	50	53.85	42.31
<i>Pseudomonas sp.,</i>	11	7.97	72.73	90.91	90.09	81.82	54.55	72.73	63.64	27.28	63.64	54.55	45.45	45.45	63.64	81.82	84.55	72.73
<i>Proteus sp.,</i>	4	2.30	75	75	0	100	25	50	50	25	50	75	25	25	50	75	50	25
<i>Acinetobacter sp.,</i>	3	2.17	0	33.33	0.	66.67	0	66.67	33.33	33.33	33.33	33.33	33.33	66.67	33.33	66.67	33.33	0
Pus (76)																		
<i>E. coli</i>	17	22.37	23.53	17.65	35.29	58.82	35.29	NA	NA	11.76	47.06	58.82	64.79	17.65	23.53	41.18	35.29	NA
<i>Klebsiella sp.,</i>	23	30.26	34.79	17.39	39.13	56.52	10.04	NA	NA	8.7	13.04	47.83	21.14	13.04	56.52	17.39	13.04	NA
<i>Pseudomonas sp.,</i>	25	32.90	40	24	60	64	24	NA	NA	12	60	40	24	28	60	32	36	NA
<i>Proteus sp.,</i>	9	11.84	66.67	44.44	66.67	55.56	22.22	NA	NA	11.11	44.44	55.56	33.33	33.33	44.44	55.56	22.22	NA
<i>Enterobacter sp.,</i>	1	1.32	100	100	100	100	100	NA	NA	0	100	100	100	100	100	100	100	NA
<i>Acinetobacter sp.,</i>	1	1.32	0	0	0	0	0	NA	NA	0	0	0	0	0	0	0	0	NA
Stool (14)																		
<i>E. coli</i>	10	71.43	20	20	30	20	30	NA	NA	0	60	50	20	60	60	60	40	NA
<i>Salmonella sp.,</i>	4	28.57	72	25	75	75	0	NA	NA	0	50	50	25	50	75	50	25	NA
Blood (49)																		
<i>Salmonella sp.,</i>	29	59.18	6.90	3.45	10.24	17.24	3.45	NA	NA	0	3.45	24.14	20.69	3.45	10.34	3.45	3.45	NA
<i>E. coli</i>	5	10.20	60	20	60	60	40	NA	NA	0	40	60	60	40	60	40	40	NA
<i>Klebsiella sp.,</i>	9	18.37	22.22	33.33	22.22	44.44	22.22	NA	NA	11.11	22.22	33.33	22.22	22.22	33.33	55.56	44.44	NA
<i>Pseudomonas sp.,</i>	2	4.08	50	50	50	50	0	NA	NA	50	0	50	50	50	50	50	50	NA
<i>Proteus sp.,</i>	2	4.08	0	0	0	0	0	NA	NA	0	0	0	0	0	0	0	0	NA
<i>Acinetobacter sp.,</i>	1	2.04	0	0	0	0	0	NA	NA	0	0	0	0	0	0	0	0	NA
<i>Aeromonas sp.,</i>	1	2.04	0	0	0	0	0	NA	NA	0	0	0	0	0	0	0	0	NA
Sputum (20)																		
<i>Klebsiella pneumoniae</i>	17	85.00	47.06	35.29	47.06	47.06	29.14	NA	NA	29.14	76.4	58.82	41.06	29.41	52.94	64.71	29.41	NA
<i>E. coli</i>	3	15.00	66.67	66.67	33.33	66.67	33.33	NA	NA	0	33.33	66.67	66.67	0	66.67	66.67	33.33	NA
Throat swab (11)																		
<i>Klebsiella pneumoniae</i>	8	72.73	37.5	12.5	50	50	25	NA	NA	12.5	87.5	87.5	50	25	50	87.5	25	NA
<i>Pseudomonas sp.,</i>	3	27.27	33.33	0	66.67	33.33	0	NA	NA	0	66.67	66.67	33.33	0	33.33	33.33	33.33	NA
Vaginal swab (5)																		
<i>E. coli</i>	2	40	50	0	0	0	0	NA	NA	0	50	50	0	50	100	50	100	NA
<i>Klebsiella sp.,</i>	1	20	0	100	100	100	0	NA	NA	0	100	100	100	0	100	0	100	NA
<i>Gardnerella vaginalis</i>	2	40	50	0	50	50	0	NA	NA	50	0	50	0	0	50	50	50	NA
Seman (5)																		
<i>E. coli</i>	5	100	0	40	40	60	60	NA	NA	0	80	80	80	40	40	20	20	NA
Catheter tip (4)																		
<i>Klebsiella sp.,</i>	2	50	100	0	100	100	50	0	0	50	100	100	100	100	50	100	100	50
<i>Pseudomonas sp.,</i>	2	50	50	0	50	0	0	0	0	50	50	50	0	0	50	50	50	0

Note: NA - Note applicable, N-Number of Isolates, %- Percentage of occurrence, G - Gentamicin, C - Chloramphenicol, CP - Cephalexin, CI - Ceftriaxone, CF - Ciprofloxacin, NA - Nalidixic Acid, NX - Norfloxacin, CFS - Cefoperazone/Sulbactam, A - Ampicillin, CA -Ceftazidime, CE - Cephotaxime, Ak - Amikacin, CB - Carbenicillin, SC - Sparfloxacin, OF - Ofloxacin, NF – Nitrofurantoin.

Antibiotic susceptibility and resistant profile for gram negative isolates varied from sample to sample as well as isolate to isolate. In which clinically important pathogens like *E. coli*, *Klebsiella sp.*, and *Pseudomonas sp.*, isolates were showed high level susceptible to Gentamicin and Cefoperazone/Sulbactam and Chloramphenicol in almost all the clinical specimens (Table 4). Similar kind of study was reported by Panta et al., [14]. Balan et al., [15] reported that amikacin found to be effective antibiotic against gram negative isolates. In our study also we found amikacin antibiotic showed appreciable effect against gram negative isolates viz, *E. coli*, *Klebsiella sp.*, *Salmonella sp.*, *Proteus sp.*, and *Acinetobacter sp.* Importantly Nitrofurantoin was found to be effective against *Acinetobacter sp.*, (100%) in urine and *Pseudomonas sp.*, (100%) in catheter tip specimens and contradictory to the findings of Vadivoo et al., [3]. Norfloxacin found to effective against *Klebsiella sp.*, and *Pseudomonas sp.*, in catheter tip specimens which supports other study reports [2] (Yadhav and Raja 2014).

E. coli was a very important and commonest organism isolated from most of the clinical specimens. The association of *E. coli* with urinary tract was well documented [2,7,16]. *Klebsiella* was the second most important pathogen obtained from urine samples. *Enterobacter sp.*, and *Acinetobacter sp.*, were found to be least common isolates from pus specimens. The in vitro effectiveness of cefoperazone/sulbactam was well proved. Cefoperazone/sulbactam (88.89%) and ciprofloxacin (77.78%) were found to be effective against the infection caused by *Proteus sp.*, in pus samples. Cefoperazone/sulbactam was the single antibiotic showed effective against *Enterobacter sp.* In children *E. coli* was observed as the major case of diarrhea and cefoperazone/sulbactam and chloramphenicol were drug of choice. *Salmonella sp.*, from blood specimens was showed sensitive to most of the antibiotics tested. *E. coli* and *Klebsiella* strains isolated from stool were observed to be sensitive to cefoperazone/sulbactam, amikacin, ofloxacin, sparfloxacin, cephotaxime and ceftazidime drugs. Similar results were observed in the study reported by Sankarankutty and Kaup [6], showed high degree of susceptibility to amikacin. *Klebsiella sp.*, *E. coli* and *Pseudomonas sp.*, were the common isolates which was predominantly isolated in other samples such as vaginal swab, semen and catheter tip. In our study *Gardnerella vaginalis* was found rarely. Colonization of catheter tip by *Klebsiella sp.*, and *Pseudomonas sp.*, was very commonly found and mainly leads to further urinary tract infection among catheterized patients. Except *Pseudomonas sp.*, remaining all other isolates were showed sensitive to cefoperazone/sulbactam, chloramphenicol, ciprofloxacin, cephotaxime and amikacin.

In case of resistant to antibiotics tested in this study *Klebsiella sp.*, and *Pseudomonas sp.*, showed high level resistance against almost all antibiotics used in this study. This was similar to the other findings [17-19].

In urine samples *Pseudomonas sp.*, showed high level resistance to nalidixic acid (72.73%), norfloxacin (63.64%) and nitrofurantoin (72.73%) antibiotics and *E.coli* showed high level resistance to norfloxacin (60.64%) and nitrofurantoin (58.51%). In pus samples *Pseudomonas sp.*, showed high level resistance to Cephalexin and Ampicillin (60%) and Ceftriaxone (64%). In sputum samples *Klebsiella sp.*, showed high level resistance to Ampicillin (76.40%). Our results is highly supported the findings of Panta et al., [14]; Javed et al., [17]; Jones [20]. Panta et al., [14] observed that *Klebsiella sp.*, and *E. coli* showed decreased susceptibility to cefotaxime and ceftriazone.

This study showed the effectiveness of nalidixic acid, norfloxacin and nitrofurantoin antibiotics were losing its capacity to kill the pathogens day by day against *E.coli* (Table 5), which supported the findings of Joseph et al., [5]. *Pseudomonas sp.*, showed higher degree of resistance to chloramphenicol, ceftriaxone and sparfloxacin (Table 5) which supported the study done by Panta et al., [14] and Yadhav and Raja [2].

CONCLUSIONS

In conclusion, it suggest that the necessity of performing antibiotic susceptibility test in hospital to profile the antibiotic susceptibility nature of each infectious organism for the correct treatment regimen. It is to report that Cefoperazone/sulbactam antibiotic could be of alternative of choice to use and to control gram-negative bacterial infection as an effective antibacterial agent. Moreover, susceptibility pattern of gram-negative isolates obtained in this study were Enterobacteriaceae showed sensitive to Cefoperazone/sulbactam antibiotic.

REFERENCES

- [1] <http://www.cdc.gov/hicpac/pdf/MDRO/MDROGuideline2006.pdf>.
- [2] Yadhav MLK and Raja A. *Int J Res Health Sci* 2014; 31; 2(3): 734-9.
- [3] Vadivoo NS, Rewa SD, Sujatha K, Niranjana M, Manivannan B, Sridevi NVK. *Global J Med Res: C Microbiol Pathol* 2014; 14(4): No 4-C.
- [4] Prashanth K, Singh SK, Kanungo R, Sharma S, Shashikala P, Joshi S, Jayachandran S. *Ind J Med Microbiol* 2010; 28(2): 130-37.
- [5] Joseph B, Sheeba SN, Sujatha S, Thanalakshmi K. *Inter J Pharmacol* 2011; 7: 463-470.
- [6] Sankarankutty J and Kaup S. *Sch J App Med Sci* 2014; 2(3A): 927-931.
- [7] Gunseren F, Mamikoglu L, Ozturk S, Yucesoy M, Biberoglu K, Yulug N, Doganay M, Sumerkan B, Kocagoz S, Unal S, Çetin S, Çalangu S, Koksall I, Leblebicioglu H, Gunaydin M. *J Antimicrob Chemother* 1999; 43: 373-378.
- [8] Cheesbrough M. *District Laboratory Practice in Tropical countries*. Cambridge University Press, London. 2000; 2: 151-4, pp. 180-265.
- [9] Mackie and McCartney. *Practical Medical Microbiology*, 14th Ed., Kundli press, Elsevier publishers, 2012, pp. 113-150.
- [10] Forbes AB, Sahm FD, Weissfelt SA. *Bailey and Scott's diagnostic Microbiology*. 12th edition. Mosby publication. 2007. 14.
- [11] Greenwood D, Slack RCB, Peutherer JF. *Medical Microbiology*. 14th edition. ELBS: 1997; pp. 781-789.
- [12] Coyle MB. *Manual of Antimicrobial Susceptibility Testing*. Washington D.C. Am. Soc. Microbiol. Press. 2005; pp. 25-39.
- [13] Gonlugur U, Bakici MZ, Ozdemir L, Akkurt I, Icagasioglu S, Gultekin F. *Annals Clin Microbiol Antimicrob* 2003; 2: 5.
- [14] Panta K, Ghimire P, Rai SK, Mukhiya RK, Singh RN, Rai G. *Asian J Pharm Clin Res* 2013; 6(1); 153-156.
- [15] Balan K, Sujitha K, Vijayalakshmi TS. *Sch J App Med Sci* 2013; 1(2): 76-79.
- [16] Vipin K, Rohit K M, Avantika C, Pramila G. *Inter J Res Pure Appl Microbiol* 2011; 1(3): 36-39.
- [17] Javeed I, Hafeez R, Anwar MS. *Biomedica* 2011; 27: 19-23.
- [18] Ghimire G, Magar JK, Bhattacharya S, Mahapatra TM. *J Ins Med* 2007; 27(2): 20-24.
- [19] Shrestha B, Basnet RB, Shrestha P, Shahi P. *J Nepal Med Assoc* 2005; 7(7): 8-11.
- [20] Jones RN. *Chest* 119 (2 Suppl). 2001; 397S- 404S.