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Isolation, biochemical characterization and antibiotic profiling of members of *Enterobacteriacea* isolated from animal fecal matter

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ABSTRACT

In the present study, fecal samples were collected from different localities of Balawala, Dehradun city. Out of 87 isolates, 50% were *E. coli*, 25% were *Klebsiella spp*., 15% were *Enterobacteriaceae spp*. and 10% were *Proteus spp*. The isolates were then checked for antibiotic sensitivity. 50% strains were resistant for novabiocin, 25% were resistant for cefixime, 15% were resistant for clotrimazole and 10% were resistant for amoxicillin and most of these showed sensitivity against the antibiotics- Amikacin, amoxicillin, cefixime, cephalexin, ciprofloxacin, clotrimazole, gentamicin, novabiocin, ofloxacin and trimethoprim. In the minimum inhibitory concentration test, 50% of the isolates showed resistance against the antibiotics amoxicillin, ampicillin, streptomycin at different concentrations (8µg/ml, 16µg/ml, 32µg/ml, 64µg/ml and 128µg/ml respectuvely) and 50% showed sensitivity against the antibiotics cefoparazone sulbactum, meropenem and piperacillin tazobactum. In conclusion, the data of the present study determine the resistance profile of enteric pathogens in animal fecal samples and is helpful from the community infection point of view. The study provides some insight on the prevalence dynamics of enteric pathogens from animal fecal which can be helpful to clinicians to formulate proper antimicrobial therapy.

KEY WORDS: AMIKACIN, RESISTANCE PROFILE, MINNIMUM INHIBITORY CONCENTRATION, MRSA, ENTEROBACTERIACEA

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INTRODUCTION

Bacterial resistance to antibiotics continues to curb our ability to treat, cure and control infectious diseases. Two organisms in particular that have become major public health threats are methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae*. Resistance to aminocyclitol aminoglycosides is an important clinical problem since these antibiotics are widely used in the treatment of serious infections, **(Larson *et al.,* 1986; Garcia *et al.,* 1989).** Large quantities of enteric bacteria from animal fecal wastes can be released into rivers and lakes that serve as sources of water for drinking, recreation or irrigation. Fecal contamination is considered to be main contributor of enteric pathogens to natural water sources. Infection originating from such sources specially diarrhea and typhoid fever. The family of *Enetrobacteriaceae* is accountable for these illnesses. The important members of *Enterobacteriaceae* are *E. coli, Salmonella* and *Shigella*. Amikacin has been the drug of choice for treating nosocomial infections refractory to other aminoglycosides **(Gerding *et al*., 1990; Levine *et al*., 1985, Kalita *et al*., 2016).**

In recent years, resistance to amikacin due to production of 3'-aminoglycoside-phosphotransferases, 2" –adenyltransferases and aminoglycoside-6'- N-acetyltransferases has been reported **(Hopkins *et al.,* 1991; Shaw *et al*., 1993; Shimizu *et al.,* 1985).** Transmission of this microbe is usually through uncooked meats and eggs. The disease is spread via the fecal-oral route and requires very low cell numbers to initiate infection. In many cases, *Shigella* infection will lead to diarrhea accompanied by fever. Among the disease caused by poultry and other farms and their products some are often severe and sometimes lethal infection such as meningitis, endocarditis, urinary tract infections, septecimia, epidemic diarrhea of adults and children. Resistance are more commonly observed among isolates of animal fecal. The relatively intensive conditions under which animal are housed may be associated with greater disease potential and therefore a greater potential and therefore a greater tendency for antibiotic use of disease control **(Bywater *et al*., 2004).**

Resistance to antimicrobials and particularly multidrug resistance is an emerging problem in Enterobacteriaceae for developing and developed countries **(Schwarz and White, 2005).** Resistant microorganisms have emerged as a result of improper use of antibiotics in human health as well as in agricultural practices **(Khachatourians, 1998).** Investigators have reported evidence of some low-level resistance to antibiotics, but overall the bacteria studied were sensitive to most antibiotics prior to exposure **(Datta and Hughes, 1983; Dancer, 1997).**

MATERIALS AND METHODS

Isolation of Enteric Pathogens: Sample was diluted appropriately in sterile saline by serial dilution method and then an appropriate dilution (0.1ml) was plated on selective media and incubated at 37 ˚C for 24 to 48 h **(Pelcezar *et al.,* 1986)** and then observed for the growth.

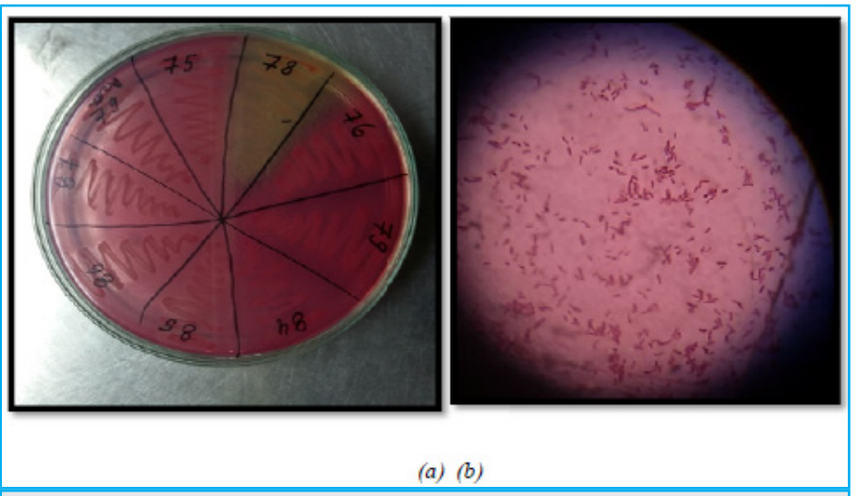
Identification and characterization of Enteric pathogens: All suspected colonies on respective selective media were presumptive forms identified using identification scheme of Bergey's manual (1997) that identifies bacteria on the basis of morphological, cultural and biochemical characteristics. The methods suggested in the microbiological methods were followed **(Borrego and Figueras, 1997)** for characterization of the bacterial isolates.

Antibiotic Susceptibility Test: Bacterial isolates viz., *E. coli, Enterobacteriaceae, Klebsiella sps., Proteus sps.* were screened for their sensitivity to antibiotics because the frequency of occurrence of these pathogens was very high. Multidrug resistant strains of these pathogens are emerging worldwide. Overnight growth of respective bacterial isolates was used for the sensitivity test. The Kirby Bauer modified disk diffusion technique was was used to determine the sensitivitity to antibiotics.The polydiscs (Micromaster Laboratories) were evenly distributed on sterile Mueller Hinton agar medium. Plates were then incubated at 37 ˚C for 24 h. The inhibition zone diameters were measured using meter scale. Inhibition zone diameters were compared with the standard inhibition zone for resistance, intermediate and susceptible character **(Kalita *et al*., 2016).**

Minimal Inhibitory Concentration (MIC): Minimum inhibitory concentration was determined according to the method described earlier by adding various concentrations of antibiotics (8-128 µg/ml) in Nutrient Broth. Further, 100 µl of inoculum was added to each tube and incubated the tubes at 37°C for 24 hours **(Sharma *et al*., 2011).**

RESULTS AND DISCUSSION

Isolation of Enteric pathogens from Animal excreta: Samples of animals were collected aseptically and transported to the laboratory immediately for isolation of enteric pathogens on Mac-Conkey agar, Eosine methylene agar, Cystine–lactose–electrolyte-deficient agar plates. The plates were incubated for 14- 16 hours *at* 37°C and after incubation observations were made there are appearances of isolated colonies. The isolated colonies were further pure cultured by sub-streaking on Mac-Conkey agar plates **(shown in Fig. 1).** The culture



(a) (b)

FIGURE 1. Isolation of Enteric pathogen(a) Selective Isolation of Enteric Pathogens on Ma Conkey Agar (b)Microscopic Observation- Gram Negative Rods

thus obtained and the details of healthy animals and humans are given in **Table 1**

In this study susceptibility pattern of pathogens liable for urinary tract infections in Poland to cogently used antimicrobial agents. A most entire study of 141 pathogens from hospital – acquired infections and 460 pathogens from community- acquired infections were isolated between July 1998 and May 1999. The most common ecological agent was *E. coli* (73.0 %), followed by *Proteus spp*. (8.9 %) and other species of *Enterobacteriaceae* (9.6 %). Few community infections were caused by Gram-positive cocci were isolated more frequently from a hospital setting (14.1 %) and the most common was *Enterococcus spp*. (8.5 %). *Pseudomonas aeruginosa* was found only among hospital isolates and was responsible for 10.7 % of infections. *E.coli* isolates from both community and hospital infections were highly affected to many antimicrobial agents with the explusion of those isolates generating elongated spectrum beta- lactamases (ESBLs). Of all *Enterobacteriaceae* tested, 38 strains (6.9 %) were able to generating ESBLs **(**Ahmed *et al*., 2011**)**.

ANTIBIOTIC SENSITIVITY TEST

Antibiotic sensitivity of all the 87 isolates was determined against 10 antibiotics belonging to β-lactam and non β-lactam group. The antibiotics included are Amikacin, Amoxycillin, Cefixime, Cephalexin, Ciprofloxacin, Clotrimazole, Gentamycin, Kanamycin, Novabiocin and Ofloxacin. There sensitivity to different antibiotics is represented in *Graph 1, 2 & 3*.

According to **Ergin & Mutlu,** 197 bacterial isolates from Sudanese patients with diarrhea or urinary tract infections. *Shigella dysenteriae* type 1 and enteropathogenic *E. coli* Showed high resistance rates against the commonly used antimicrobial agents: ampicillin, chloramphenocol, amoxycillin, co-trimoxazole, tetracycline, malidixic acid, sulfonamide and neomycin. The uropathogens wre completely sensitive to ciprofloxacin. Resistance to tetracycline, amoxicillin, ampicillin, cotrimoxazole and sulfonamide was the most frequent pattern. The common urinary tract pathogens *Klebsiella pneumonia, E. coli* and *Proteus mirabilis* showed high rates of resistance to ampicilin, co-trimoxazole, amoxicillin, tetracycline, trimethoprim, sulfonamide, streptomycin and carbenicillin.

MINIMUM INHIBITORY CONCENTRATION

Of all 87 samples 25 samples were selected for carrying out MIC of Amoxycillin, Ampicillin, Pipracillin tazobactum, Streptomycin, Meropenem and Cefoparazone sulbactum. The MIC was conducted at different concentrations like (8µg, 16µg, 32µg, 64µg and 128µg). Maximum isolates showed resistance against Amoxycillin and minimum against Meropenem and Cefoparazone sulbactum. In decresing order of resistance antibiotics can be placed as Amoxycillin>Ampicillin>Streptomycin >Pipracillintazobactum>Meropenem>Cefoparazone sulbactum. The MIC result of isolates is shown in *Graph. 4, 5, 6 & 7*.

In this study they determined the distribution rates of *Pseudomonas aeuroginosa* in clinics and its resistance to antibiotics. The antibiotic resistance rates were detected by minimal inhibitory concentration (MIC). The clinical and specimen distribution properties of *Pseudomonas* were evaluated based on their resistance pattern. *Pseudomonas* was the fourth common bacteria in all isolates.

Table 1. Cultures obtained from Animal Fecal Matter

S. No	Sample Number	Growth On MacConkey Agar	Morphology	Motility
1.	AH1	Small pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
2.	AH2	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
3.	AH3	Pink color colnies/ pink background	Small pink color colonies and mucoid	-ve
4.	AH4	Pink color colonies/ pink background	Small pink color colonies and mucoid	-ve
5.	AH5	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
6.	AH6	Pink color colonies/ pink background	Small pink color colonies and mucoid	-ve
7.	AH7	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
8.	AH8	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
9.	AH9	Pink color colonies/ pink background	Small pink color colonies and mucoid	-ve
10.	AH10	Yellow swarming colonies/ yellow background	Yellow color colony and show motility	+ve
11.	AH11	Small pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
12.	AH12	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
13.	AH13	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
14.	AH14	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
15.	AH15	Yellow color colonies/ yellow background	Gram –ve, non-motile and rod shaped	-ve
16.	AH16	Colorless colonies/ white background	Gram –ve, non-motile and rod shaped	+ve
17.	AH17	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
18.	AH18	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
19.	AH19	Translucent gummy colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
20.	AH20	Colorless colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
21.	AH21	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
22.	AH22	Pink color colonies/ pink background	Small pink color colonies and mucoid	-ve
23.	AH23	Translucent gummy colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
24.	AH24	Colorless colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
25.	AH25	Yellowish gummy colonies/ yellow background	Gram –ve, non-motile and rod shaped	-ve
26.	AH26	Colorless colonies/ white background	Gram –ve, non-motile and rod shaped	+ve
27.	AH27	Colorless colonies/ white background	Gram –ve, non-motile and rod shaped	+ve
28.	AH28	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
29.	AH29	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
30.	AH30	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
31.	AH31	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
32.	AH32	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
33.	AH33	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
34.	AH34	Pink color colonies/ pink background	Small pink color colonies and mucoid	-ve
35.	AH35	Colorless colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
36.	AH36	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
37.	AH37	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
38.	AH38	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
39.	AH39	Pink color colonies/ pink background	Small pink color colonies and mucoid	-ve
40.	AH40	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
41.	AH41	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
42.	AH42	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
43.	AH43	Colorless colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
44.	AH44	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve

44.	AH44	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
45.	AH45	Colorless colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
46.	AH46	Colorless colonies/ pink background	Gram –ve, non-motile and rod shaped	-ve
47.	AH47	Translucent gummy colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
48.	AH48	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
49.	AH49	Small orange color colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
50.	AH50	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
51.	AH51	Colorless colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
52.	AH52	Pink color colonies/ pink background	Small pink color colonies and mucoid	-ve
53.	AH53	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
54.	AH54	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
55.	AH55	Translucent gummy colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
56.	AH56	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
57.	AH57	Translucent gummy colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
58.	AH58	Colorless colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
59.	AH59	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
60.	AH60	Colorless colonies/ pink background	Gram –ve, non-motile and rod shaped	-ve
61.	AH61	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
62.	AH62	Yellow swarming colonies/ yellow background	Yellow color colony and show motility	+ve
63.	AH63	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
64.	AH64	Colorless colonies/ pink background	Gram –ve, non-motile and rod shaped	-ve
65.	AH65	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
66.	AH66	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
67.	AH67	Yellow swarming colonies/ yellow background	Yellow color colony and show motility	+ve
68.	AH68	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
69.	AH69	Colorless colonies/ pink background	Gram –ve, non-motile and rod shaped	+ve
70.	AH70	Translucent gummy colonies/ white background	Gram –ve, non-motile and rod shaped	+ve
71.	AH71	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
72.	AH72	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
73.	AH73	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
74.	AH74	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
75.	AH75	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
76.	AH76	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
77.	AH77	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
78.	AH78	Translucent gummy colonies/ yellow background	Gram –ve, non-motile and rod shaped	+ve
79.	AH79	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
80.	AH80	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
81.	AH81	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
82.	AH82	Pink color colonies/ white background	Small pink color colonies and mucoid	+ve
83.	AH83	Colorless colonies/ white background	Gram –ve, non-motile and rod shaped	+ve
84.	AH84	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
85.	AH85	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
86.	AH86	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve
87.	AH87	Pink color colonies/ pink background	Small pink color colonies and mucoid	+ve

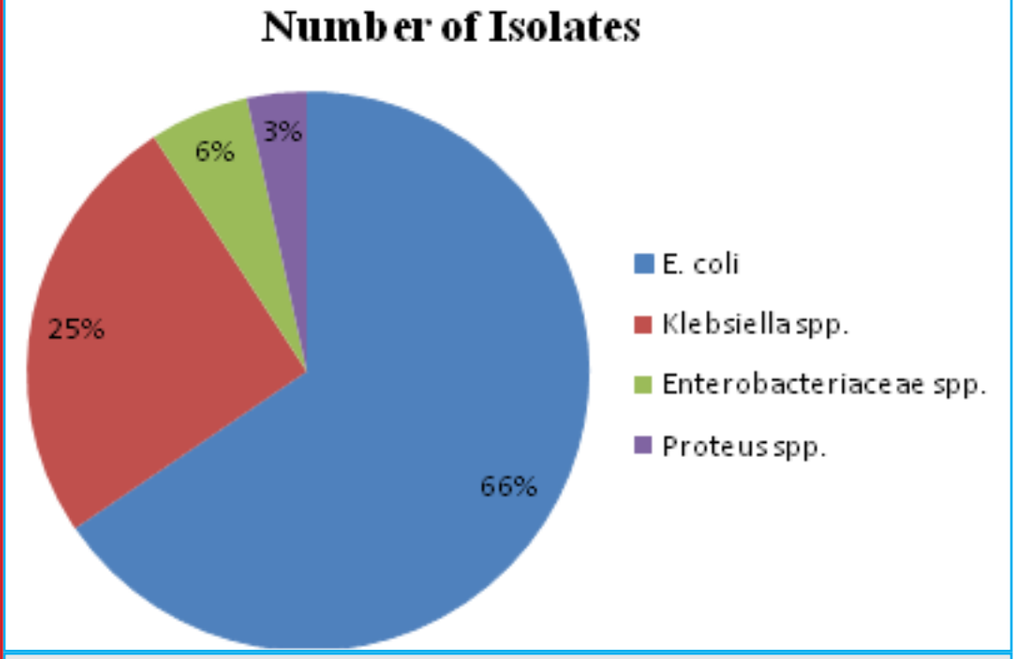
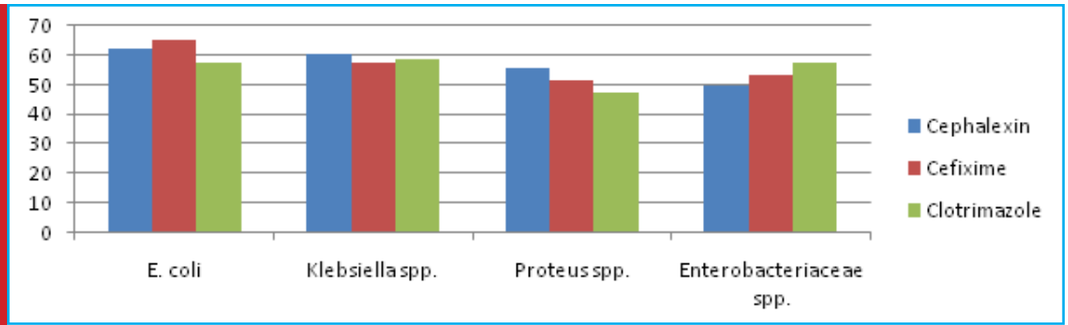
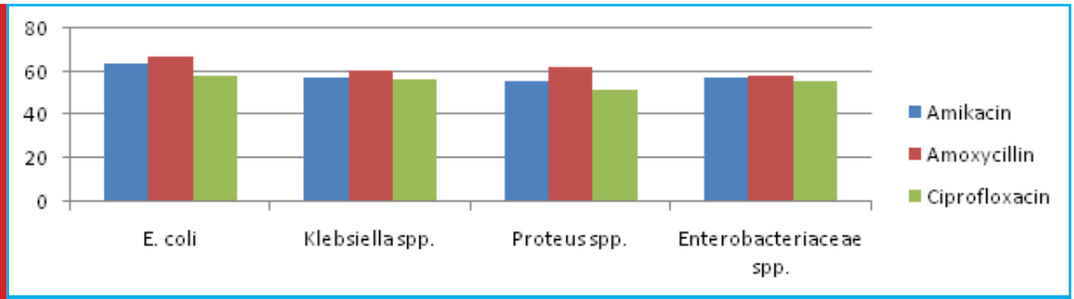


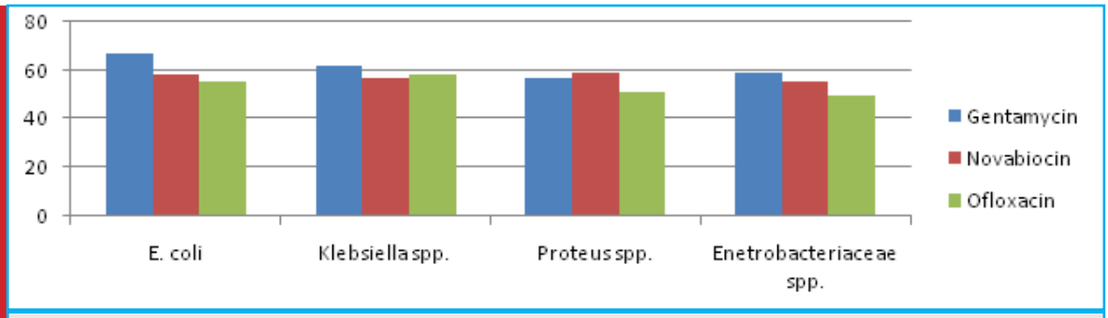
FIGURE 2. Cultures obtained from Animal Fecal Matter



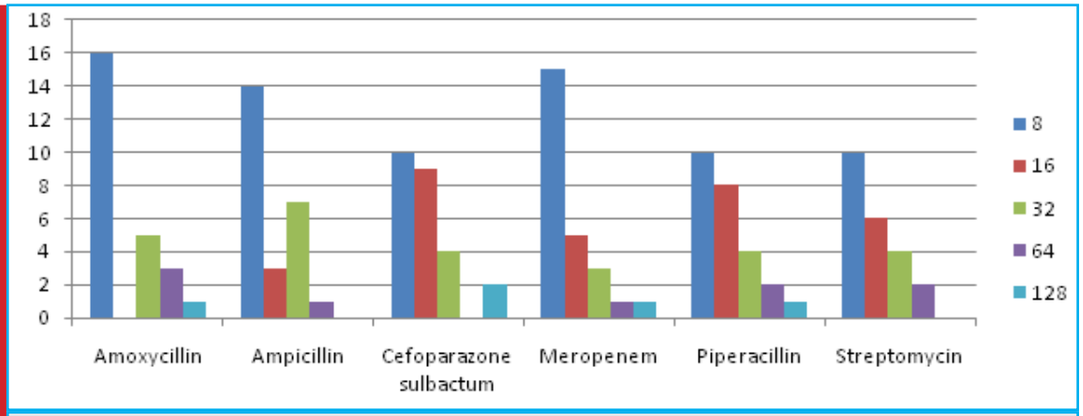
GRAPH 1. Antibiotic Sensitivity Of Selected Strain To β-Lactam Antibiotics



GRAPH 2. Antibiotic Sensitivity Of Selected Strain To Non β-Lactam Antibiotics



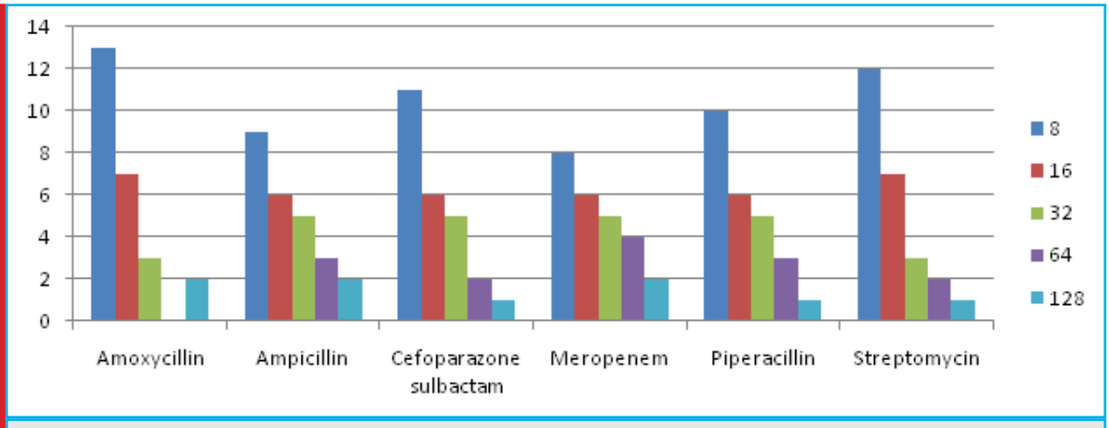
GRAPH 3. Antibiotic Sensitivity Of Selected Strain To Non β-Lactam Antibiotics



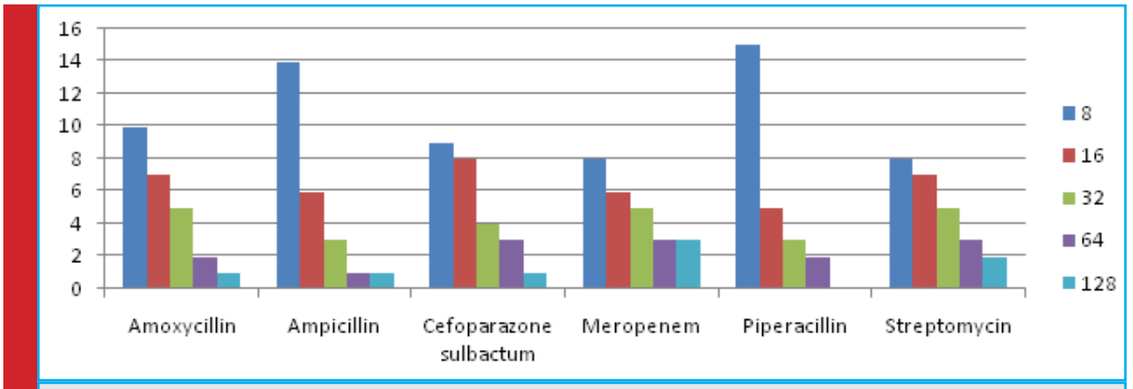
GRAPH 4. MIC Concentration Against Different Antibiotics for E.Coli

Tracheal aspirates, sputum and wound, pus were important sources for *Pseudomonas aeruginosa* isolation in intensive and nonintensive care units of surgery wards (SW-ICU, SW-nonICU) ($p<0.05$). on the basis of MIC criteria, the resistance ratios of the isolates to cefriaxone, cefotaxime, ceftazidime, imipenem, ofloxacin and ciprofloxacin were 8.4%, 15.0%, 13.3%, 0.0%, 11.6 % and 8.3% respectively **(Hryniewicz *et al.*, 2001)**.

A wide range of pathogenic microorganisms can be transmitted to humans via water contaminated with fecal matter. These include enteropathogenic agents such as *E. coli, Shigella, salmonella, enteroviruses* and multicellular parasites as well as opportunistic pathogens like *Pseudomonas aeruginosa, Klebsiella* etc. Applications of antibiotics bring about an increase in resistance to antibiotics not only in pathogenic bacterial



GRAPH 5. MIC Concentration Against Different Antibiotics for Enterobacteriaceae sps.



GRAPH 6. MIC Concentration Against Different Antibiotics for Klebsiella sps.

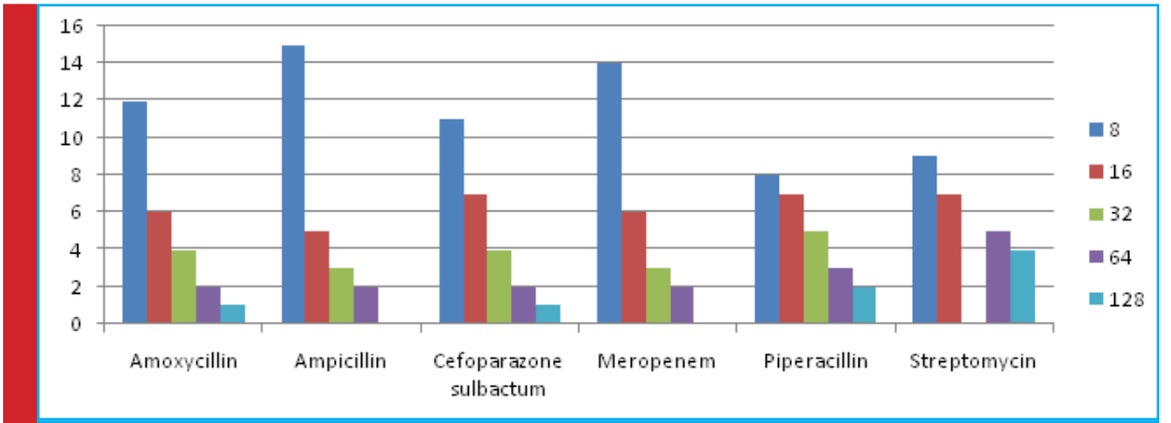
strains, but also in commensal bacteria **(Luzzaro *et al.*, 2001)**

In the present study, the samples from different localities of Balawala were collected and total of 87 isolates were obtained from them and among these 50% were *E. coli*, 25% were *Klebsiella* spp., 15% were *Enterobacteriaceae* spp., and 10% were *Proteus* spp. The isolates were then identified on the basis of biochemical characteristics and the *Klebsiella, E. coli, Proteus, Enterobacteria* and *Pseudomonas* were isolated from the excreta of animals. Antibiotic resistance among the isolates was also evaluated using for antibiotics- amikacin, gentamicin, novabiocin, ofloxacin, ciprofloxacin, cephalexin, cefixime, amoxicillin, clotrimazole, trimethoprim, kanamycin, ampicillin, streptomycin, meropenem, piperacillin tazobactam and cefoparazone sulbactum.

In our study it has been seen that resistance was seen for novabiocin (50%), cefixime (25%), clotrimazole (15%) and amoxicillin (10%). It was also found to be sensitive for gentamicin, amikacin, kanamicin, trimethoprim, ciprofloxacin and ofloxacin. The MIC test was also conducted during this study those isolates are chosen for the MIC that showed more resistance efficacy. The MIC has been performed by chosing the different isolates in which following antibiotics was used viz amoxicillin, ampicillin, cefoparazone sulbactum, meropenem, piperacillin tazobactum and streptomycin. 50% of the isolates showed resistance among the antibiotic amoxicillin, ampicillin, streptomycin at different concentrations (8µg/ml, 16µg/ml, 32µg/ml, 64µg/ml and 128µg/ml) and 50% showed sensitivity against the antibiotic cefoparazone sulbactum, meropenem and piperacillin tazobactum. The high density of enteric pathogen and prevalence of multidrug resistant *E. coli, Proteus* and *Kleibsiella* in the fecal matter may pose severe public health risk.

CONCLUSION

In this study, we analysed the susceptibility pattern of different aminoglycosides in different locality of Bala-



GRAPH 7. MIC Concentration Against Different Antibiotics for Proteus sps.

wala, strain collections of *E. coli*, *Klebsiella spp*., *Pseudomonas spp*., *Enterobacteriaceae spp*. and *Proteus spp*. Enteric pathogens, which are of great concern since they are the most common causes of infection among humans and animals. Aminoglycosides represent an important class of antimicrobial agents. The prevalence of aminoglycoside resistance among Gram-negative bacteria in Dehradun is low, but an increased prevalence among clinical isolates of *Escherichia coli* has been observed during the last years. The most prevalent resistance mechanism is aminoglycoside modifying enzymes.

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