ANTIMICROBIAL RESISTANT *ESCHERICHIA COLI* IN A SUB-URBAN COMMUNITY OF KATHMANDU VALLEY

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ABSTRACT

In this study total 68 samples including water, chicken faeces, children stool samples were collected in Macche Narayan VDC in four seasons for a year. Altogether 53 different bacterial isolates were isolated from those samples. Among them 37 isolates belonged to *E. coli* and rest of them were the member of different gram negative rods. Among the total isolates 56.66% were resistance to one or more than one antibiotics. Among the total *E.coli* 32% were resistance to one a more than one antibiotic. MICs and MBCs values of these isolates (i.e resistant *E.Coli)* against Amplicilling and Tetracycline were determined. The MICs were 1000 μg/ml and 125 μg/ml and the MBCs values were 2000 μg/ml and 500 μg/ml against Ampicillin and Tetracycline respectively.

INTRODUCTION

The increasing prevalence of bacterial resistance to most antibiotics is of concern as the choice of agent available for treatment becomes limited and inevitabilly more complicated. Furthermore, the potential exists where no available therapeutic agent would be effective against many pathogens (Murray et al., 1990). Bacteria can develop large varieties of mechanisms for the antibiotic resistance and they possess genetic mechanism for the spread of this resistant to every new antibiotics. The discovery by Japanese workersm 1959 describes that the multiple resistance of Shigella strain could be transferred to other member of the Enterobacteriaceae by conjugation (Anderson, 1965, Datta, 1965).

The incidence of antibiotic resistance among clinical isolates is well documented Antimicrobial drug resistance has been shown to be higher in developing world rather than the developed world (Farrer, 1985). Trimethoprin resistance among pathogenic Gram negative bacteria was found to be 64% in South India (Young et al., 1986), 49.1% in South Africa (Wylie & Koornhad, 1989) and 63.3% in Nigeria (Lamikanra & Ndep, 1989). Reasons for the high levels of resistance in the developing countries may include the availability of antibiotics without prescription and contamination of water supply (Amyes et. al., 1992). Bacteria isolated from a variety of aquatic and terrestrial habitats have also demonstrated resistance to antimicrobial agents. Generally, bacteria with

the greatest levels resistance have been isolated from hospital wastes with significant contamination by antimicrobial agents (Datta, 1969, Fountain & Hoadley, 1976) and drinking water (Armstrong et al., 1981).

Escherichia coli is a part of normal bacterial flora of gut although there do exist strains of *E. coli* (Enterotoxigenic, Enteropathogenic, Enteroinvasive) that cause diarrohea. *E. coli* diarrhoea commonly occurs among children resulting in severe distress. Therapeutically, in this area of study, there is often prevailing tendency to mediate the children with antibiotics, perhaps to shorten the duration and severity of the infection. This approach is often indulged in with disregard to the antibiotic adverse consequences before seeking medical attention, although just oral dehydration (WHO, 1980).

One of the dangers of self-medication with antibiotics in cases of acute diarrhea is the selection of gut bacterial strains with multiple antibiotic resistance Enteropathogenic *E.coli* (EPEC) strains have been reported to co-transfer enterotoxigenic with R-plasmids conferring resistance to several antibiotics (Rotimi, et al. 1984). The normal commensal is increasingly recognized as a reservoir of antibiotic resistance. The level of resistance in this group appears to be higher in developing countries rather than developed countries. This study examines resistant *E. coli* in community of Sub-urban community of Kathmandu valley.

Nepal is a developing country. Most of the people are illiterate & do not know antibiotics and resistant transfer mechanisms in bacteria. So, they are not aware of effect of irrational use of drugs, wrong dose and dose taken insufficient length of time. The selection of drug and information about drug resistance are not communicated to those prescribing antimicrobials. The resistant antimicrobial agents have been still on use of infectious diseases. Hence, the microbial resistance is posing a problem to treat infectious disease caused by resistant pathogens of genetic origin and transferable bacterial species and genera.

MATERIALS AND METHODS

The samples (water, chickens faeces, children stool) were collected near to Kirtipur municipality. Two community ponds located in Macche Naraya VDC Ward No. 1,3 and 5 were selected for this study. There are approximately 850 houses and population about 5500. Water samples from ponds and water storage pots, chickens faeces and children stool were collected from two ponds and five selected also from houses. This study was conducted during a period of a months (Sept. to Dec. 1997.) The sampling interval was of one month.

SAMPLE COLLECTION, ISOLATION AND IDENTIFICATION

Water samples were collected in sterilized sampling bottles and transport as soon as possible to the laboratory. This water sample was examined by membrane filter technique having pore size 0.45 up for total coliform count in

M-endo agar and also enumerated the total coliform count by pour plate technique in Full name XLD medium. A microbial count can be made before and after a specific treatment and results obtained indicate the degree to which the bacterial population has been reduced.

Chicken faeces and children stool samples were directly inoculated on XLD media and isolation of different family of Enterobacteraceae was performed.

Diferent bacteria isolates were identified by following standard microbiological methods using differential media as described in Bergey's manual of systematic Bacteriology (1986) identified different bacteria isolates.

STUDY OF ANTIBIOTIC RESISTANT PATTERN

Antibiotic sensitivity patterns of bacterial isolates were determined by Kirby Bauer method (Bauer et al. 1966). The following antibiotic discs of different concentration: Ampicillin (10 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Nalidixic acid (30 µg), Streptomycin (10 µg), Norfloxacin (10 µg), Nitrofurantoin (30 µg), Kanamycin (30 µg) and Gentamycin (30 µg) were used during experiment. After the detection of one or more than one antibiotic resistant by Kirby Bauer Method, the Minimum Inhibitory Concentrations (MIC) and Minimum Bacterial Concentrations (MBCs) were also determined for the resistant *E. coli* following standard protocol (....) . The Minimum Inhibitory Concentrations (MICs) was considered to be the lowest concentration of antibiotic which produced no significant growth. The growth differences between control and culture grown on nutrient broth. For Minimum Bacterial Concentration isolates were selected from all tube showing no significant growth. From these tubes isolates were subculture on to the Agar medium MBCs of resistant *E. Coli* was determined.

RESULTS

Water samples were collected from two ponds (upper and lower) and water storage pots of five different houses of Macche Narayan VDC. These seven samples were examined for bacteriological study during four different months. The total coliform count was 91 CFU/ml in the month of December in lower pond. For, water storage pots the highest total coliform count (29 CFU/ml) was counted in month of December in house No. II as shown in Fig. - 1.

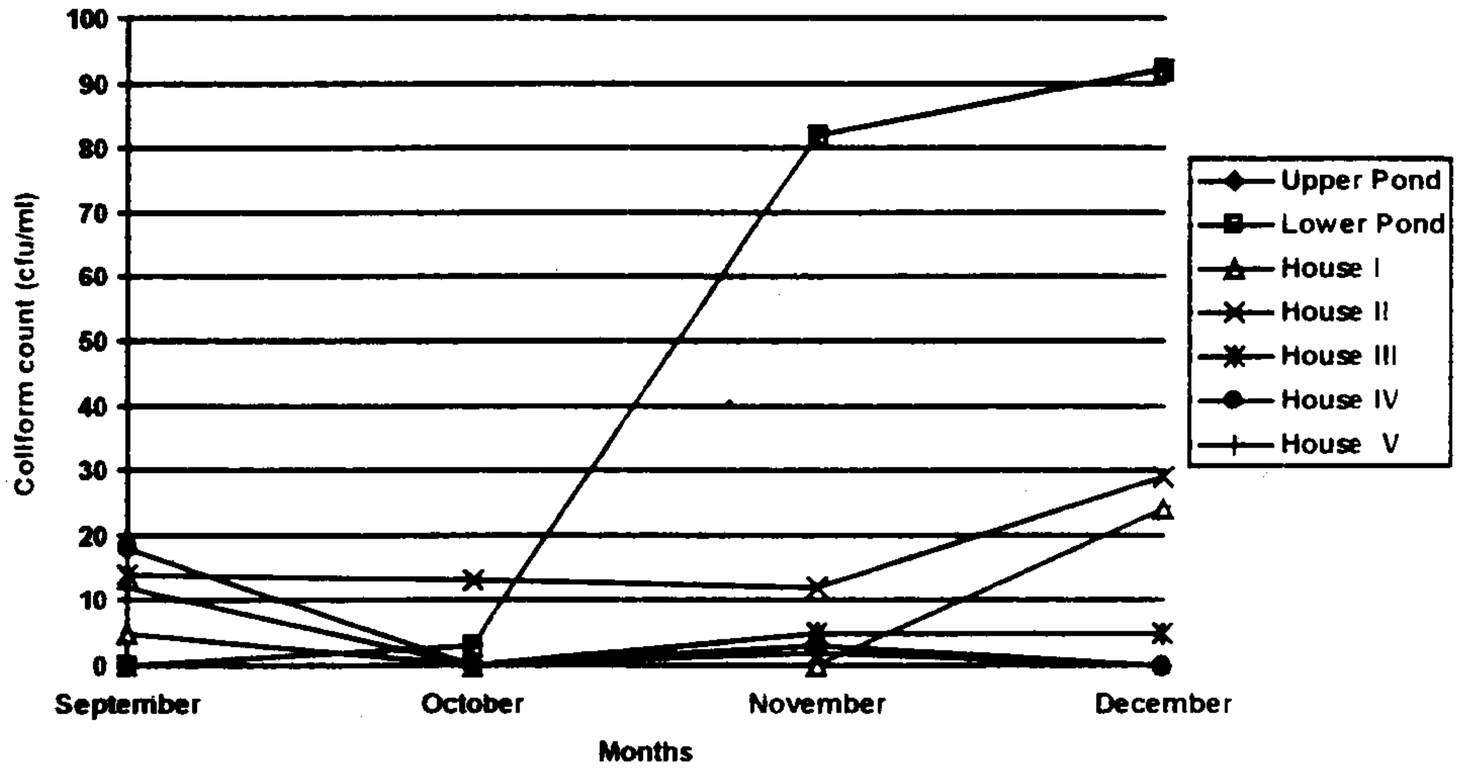

Figure -1: Graph showing total coliform count in the water samples in different months from pond water & storage pots water samples.

Among the total 53 isolates from water, chicken faeces and children stool samples, 69.81% was found to be *E. cole* and 30.19% was other gram negative bacteria, specially belonging to *Salmonella spp., Shigella spp., Klebsiella spp. Enterobacter spp.* etc. These isolates were 100%, 98.11%, 92.45%, 96.22%, 96.223%, 26.4%, 90.56%, 94.33% and 79.24% sensinve to Gentamycin (30 μg), Norflosacin (10 μg), Chloramphenicol (30 μg), Nalidixic acid (30 μg), Kanamycin (10 μg), Ampicillin (10 μg), Tetracycline (30 μg), Streptomycin (10 μg) and Nitrofurantoin (30 μg) respectively.

Biochemical identification showed that 37 isolates were *E. coli,* and their pattern towards Antitrionics sensitivity.as judged by Kirby-Bauer Method (Bauer et al, 1966) have been shown in Table 1.

Table- 1: Resistance Pattern of *E.coli* towards one or more Antibiotics.

Percentage of Resistively towards Antibiotics	A	T	N	C	St.	K	G	Nf	Ni
1.	42.24	13.51	2.70	2.70	5.40	5.40	0	0.53	24.32

Note:- A= Ampicillin, T=Tetracycline, N=Nalidixic acid, C= Chloramphenicol, St=Streptomycin, K=Kanamycin, G=Gentamycin, Nf= Norfloxacin, Ni= Nitrofurantoin .

Minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) values of *E.coli* isolates to Ampicillin and Tetracycline were determined. Seventeen out of 37 *E. coli* isolates were dejected to be resistant against all the antibiotics tasted. MICs and MBCs value were determined for all the 17 resistant isolates of *E.coli* against Ampicillian and Tetracycline and the obtained have been presented results in Table 2.

Table- 2: MICs and MBCs Values of Ampiallin & Tetracycline on Different Resistant *E.coli* .

S. No.	Code No.	Amplicillin (μg/ml)		Tetracyclines (μg/ml)	
		MICs	MBCs		
1.	KMSW4	7.815	31.25		
2.	$KMSW_5$	62.5	500.0		
3.	$KMSC_1$	62.5	250.0		
4.	$KMSC_3$	31.25	125.0		
5.	$KMSC_4$	31.25	250.0	125.0	500.0
6.	$KMSH_1$	31.25	250.0		
7.	$KMSH_3$	62.5	500.0		
8.	$KMOW_2$	31.23	500.0		
9.	$KMOC^3$	125.0	1000.0		
10.	$KMNH_4$	31.25	500.0	31.25	250.0
11.	$KMNH_2$	7.815	31.25		
12.	$KMNH_4$	62.5	500.0		
13.	$KMDW_5$	7.815	62.5		
14.	$KMDW_4$	1000.0	2000.0	15.25	125
15.	$KMDC_3$	125.0	1000.0	62.50	250
16.	$KMDC_5$	7.815	125.0		
17.	$KMDH_3$	1000.0	2000.0		

The above results indicate the MICs values of Amitricillian against resistant. *E. coli* isolates was 1000 μg/ml and MICs value of Tetracycline was 125 μg/ml. The highest MBC value was 1000 μg/ml, and 500 μg/ml for Amplicillin and Tetracycline respectively.

DISCUSSION

This study was undertaken to determine the drug resistant *E. coli* in community. Water is a vital resource for human, but it can be extremely dangerous when it become vehicle of transmission of diseases and can cause explosive outbreaks of such diseases, which may be widely disseminated. Many studies have implicated the poor water quality as a principle factor for mortality and mortality associated with enteric diseases. Similarly, the cause of typhoid, cholera and gastrointerititis illness were found closely related to poor water quality. Many of them from diseases attributable to a lack of safe water or adequate sanitation facilities. When these pathogens become epidemic, then the whole community will be panic.

Microbiological analysis of water sample from two ponds and water storage pots from five different houses showed that the total coliform count in lower pond was found to be higher than in the upper pond which could be due to human activity, faecal matters from baby diapers, kitchen wastes and animal excreta accumulated by the public during washing and cleaning. High coliform count in the lower pond which was the maximum (91 CFU/ml) in the month of December (Fig. – 1). This is comparatively low value as compared to ponds of Kirtipur which had average of 2×10^4 CFU/Ml, (K.C., et al., 1997) an a also lower than coliform count in Phewa Lake, which was 4,800 CFU/ml (CEDA, 1989). The presence of high coliform bacteria and *E. coli* in water sample is the indicator of faecal contamination i.e. high number indicates unsatisfactory sanitary practices and poor hygienic condition of natural water sources.

In water sample, 18 total isolates were isolated. Among them 10 (55.55%) were *E. coli* and rest 8 (45.45%) were other members of Enterobacteraceae such as *Emerobacter spp., Klebsiella spp., Salmonella spp., Shigella spp. Proteus spp.* Among then total *E. coli* isolates (50%) were resistance to Ampicillin and Tetracycline. This results agreed with that there of Marigo et al. (1990; Bel et al., 1980; and Alther et al., 1982 WHO had reported the antibiotic resistant Salmonella and coliform groups of bacteria occurring in natural water and sewage effluents. Likewise, Sharma (1993) isolated *E. coli, Salmonella spp., Klebsiella spp., proteus spp.* and *Shigella spp.* from water of different parts of the country.

MICs and MBCs values against resistant *E. coli* Ampicillin and Tetracycline were determined. Result shows that the MICs value (1000 μg/ml)

and MBCs value (> 2000 μg/ml) were observed against Ampicillin, whereas in case of Tetracycline MICs was 125 μg/ml and MBCs value was > 500 μg/ml (Table – 2). Such high MIC and MBC are remarkable in the context of Nepal.

This microbiological studies showed that the community studied were highly contaminated with different group of bacteria. There is high number of antibiotic resistant bacteria, which can play a crucial role in public health. If these antibiotic resistant bacteria were found to possess resistant transfer mechanisms, inside such community a lot of sensitive bacteria can be changing into resistant bacteria by this mechanism and could have spread in surrounding communities causing remarkable problems.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Paul Shears, Director, Centre for Tropical Medial Microbiology, The University of Liverpool for his valuable suggestions and necessary help during this research.

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