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ISOLATION AND CHARACTERIZATON OF MULTIDRUG RESISTANT *ESCHERICHIA COLI* FROM HOSPITAL, DAIRY AND STUDENT HALL WASTES

NAZMUN NAHER, MD ZOBAIDUL ALAM AND A.M.MASUDUL AZAD CHOWDHURY[*1]
Department of Microbiology, [1]Department of Genetic Engineering and Biotechnology,
University of Chittagong, Chittagong -4331, Bangladesh.

ABSTRACT

In this investigation a total of 31 isolates of *E. coli* were isolated randomly from different samples to analyze the resistance pattern to the most frequently used antibiotics. Among these 31 isolates 17 were isolated from hospital, 9 from student hall and 5 from dairy farm wastes. The isolates from hospital wastes showed 100% resistance to ciprofloxacin and ampicillin, 94% to penicillin, 76% to tetracycline, 41% to gentamicin and 35% to chloramphenicol. In contrast, the isolates from dairy farm wastes showed 100% resistance to penicillin and ampicillin, 80% to ciprofloxacin, and 40% to tetracycline. On the other hand, among the isolates of hall wastes 66% showed resistance to penicillin and ampicillin, 55% against ciprofloxacin and 44% to tetracycline. However all the isolates of dairy farm and hall wastes were sensitive to gentamicin and chloramphenicol.

Key words: Antibiotic, ciprofloxacin, *Escherichia coli*, multidrug resistant, wastes.

INTRODUCTION

It is well known that infectious diseases account for high proportion of health problems, specially in the developing countries due to inefficient health management practices. In low-resource countries the impact of antimicrobial drug resistance on illness and death rates tends to be greater because of the high prevalence of bacterial infections and the major role of antimicrobial agents in combating infectious diseases *(*Col 1987, Brien 1992, Okeke *et al*. 1999, World Health Organization 2001, Byarugaba 2004). The high antimicrobial drug resistance rates observed in low-resource countries are likely due to a combination of several factors, among which irrational antimicrobial drug use and

[*]Corresponding author:

conditions of poor sanitation are thought to play a major role, even if the relative importance of additional factors remains unclear *(*Okeke *et al*. 1999, Walson *et al*. 2001 Byarugaba 2004, Bartoloni *et al*. 2006,). Microorganisms have developed resistance to many antibiotics and thus has created immense clinical problem in the treatment of infectious diseases (Davis 1994). This raises the importance to investigate the involvement of hospital and dairy farm liquid waste discharges in the development and distribution of antibiotic resistance in environmental bacteria including enterobacter such as *E. coli* (Mathew *et al*. 1998, Albert *et al*. 2000, Guerra *et al*. 2003, Al-Tawfiq 2007). Both samples bear the correlation of antibiotic use, poor waste disposal and antibiotic resistance in development. *E. coli*, the most significant species in the genus *Escherichia*, is recognized as an important potential pathogen in human. Being a gram-negative bacillus, it is a common isolate from the colon flora. Although most strains are harmless, several are known to produce toxins that can cause diarrhoea. *E.coli* has been associated with a wide variety of diseases and infections, including meningeal (predominantly in the newborn), gastro intestinal, urinary tract, wound and bacterimic infections in all age groups (Matsuo *et al.* 2002). The present experiment was undertaken to find out how and in what proportion the hospital, dairy and student hall wastes are distributing the resistance phenomenon among the bacteria *E. coli* against the different broad spectrum antibiotics.

MATERIALS AND METHODS

Sample Collection

Liquid wastes were collected from the septic tank (sample1) and sewage drain (sample 2) of Chittagong Medical College Hospital (CMCH), dairy samples (Sample 5) were collected from Bangladesh Agricultural Research Institute (BARI), Hathajari, Chittagong. Other samples collected directly from the septic tank (Sample 3) and outlet drain (Sample 4) of Samsunnahar Hall (SH), Chittagong University, Chittagong.

Isolation and identification

The samples were diluted with sterile saline. Sample suspensions were incubated using nutrient agar plate at 37°C for 24 hours. Single colonies were picked up by sterile tooth picks randomly from the NA plates and then streaked on Eosine Methylene Blue (EMB) agar plate. The plates were incubated at 37°C for 24 hours. In EMB plate typical *E. coli* is usually characterized by green

metallic sheen colony. After incubation, the colonies (single) which were close to geve *E. coli* characteristic picked up and coded. Conventional biochemical tests used for the identification of *E. coli* are presented in table 1.

Antibiotic bioassay

Antibiotic sensitivity test of the isolates were conducted by disc diffusion method (Bauer and Kirby 1966) in Mueller Hinton Agar medium with disks containing chloramphenicol (30 µg), ampicillin (10 µg), penicillin (10 µg), tetracycline (30 µg), gentamicin (10 µg) and ciprofloxacin (50 µg).

RESULTS AND DISCUSSIONS

A total of 31 individual colony of *E. coli* (17 from the Hospital, 9 from Samsunnahar Hall and 5 from BARI waste samples, respectively) were isolated and characterized according to the biochemical properties. The results of the various tests associated with *E.coli* isolates are listed in table 1. All of them were found positive to lactose fermentation, formation of indole and MR test, but negative to VP test, citrate test and urease test. The results of cultural and biochemical tests confirmed that the isolates were *E. coli.*

The resistance patterns of the isolated *E. coli* strains are presented in table 2. Most of the isolates of hospital, dairy and hall wastes were found resistant to penicillin, ampicllin, ciprofloxacin, tetracycline, gentamicin and chloramphenicol. Multi drug resistance in *E.coli* is not uncommon because it was reported that a multidrug resistance regulatory chromosomal locus is wide spread among enteric bacteria (Cohen *et al.*1993). High percentages of multidrug resistant isolates were observed in liquid hospital waste because antibiotics are used largely by the practitioners. This high occurrence of resistance might be the result of extensive use of antibiotic among hospital patients. The emergence of antibiotic resistant bacteria in hospital wastes may be due to their flourish in the patient's intestines, or increasing use or misuse of the particular antibiotics against which the resistance developed (Blondeau *et al*. 1996, Acar *et al*. 1997, Richard *et al*. 1994). Again, antibiotics are used in agriculture as supplements to animal feeds both as growth promoting substances and as prophylactic additives to prevent the occurrence of disease rather than to treat an actual infection.

TABLE 1: BIOCHEMICAL REACTION OF DIFFERENT ISOLATES

Name of the Isolates from different samples	Indole production	Methyl-red test (MR)	Voges-Proskauer	Simmons citrate	Urea hydrolysis	Motility (36^0)	Lactose fermentation	Mannitol fermentation	Oxidase test	Glucose fermentation	EMB agar plate metallic sheen colony	Macconkey agar plate	Name of isolates
Sample-1													
PS_1R_1	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_1R_2	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_1R_3	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_1R_4	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_1R_5	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_1R_6	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_1R_7	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_1R_8	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_1R_9	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_1R_{10}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_1R_{11}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
Sample-2													
PS_2R_{12}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_2R_{13}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_2R_{14}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_2R_{15}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_2R_{16}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_2R_{17}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
Sample-3													
PS_3R_{18}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_3R_{19}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_3R_{20}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_3R_{21}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_3R_{22}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
Sample-4													
PS_4R_{23}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_4R_{24}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_4R_{25}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_4R_{26}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
Sample-5													
PS_5R_{27}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_5R_{28}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_5R_{29}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_5R_{30}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*
PS_5R_{31}	+	+	-	-	-	+	+	+	-	+	+	+	*E.coli*

TABLE 2**:** PATTERN OF RESISTANCE OF *ESCHERICHIA COLI* ISOLATES FROM HOSPITAL, HALL AND DAIRY WASTES

Antibiotics	Diffusion Zone break points (mm)	Hospital isolates (%) (n=17)			Hall isolates (%) (n=9)			Dairy isolates (%) (n=5)		
		S	I	R	S	I	R	S	I	R
Gentamicin	≤12	52	7	41	100	0	0	100	0	0
Penicillin	≤13	6	0	94	34	0	66	0	0	100
Ampicillin	≤13	0	0	100	23	11	66	0	0	100
Chloramphenicol	≤12	35	30	35	88	12	0	100	0	0
Ciprofloxacin	≤15	0	0	100	44	0	56	20	0	80
Tetracycline	≤14	18	6	76	44	12	44	40	0	60

S:Sensative R:Resistant I:Intermediate

It was observed from the present study that higher percentage of antibiotic resistance were shown by the *E coli* isolates from the hospital wastes while dairy farm waste showed intermediates and student hall wastes had the minimum. The result was indicative of the use and misuse of antimicrobial agents by different users. Extensive uses of antibiotics contribute greatly to the emergence of antibiotic resistant microorganisms in the environment which has significant negative impact in the treatment of disease.

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