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Antibiotic susceptibility profiling and in-vitro antibacterial activity of some plant extracts to *Escherichia coli* isolated from spoiled rice and egg

M. Javed Foysal[1], M. Nazmul Hossain[1], Fahmida Yeasmin[2], Shamsul Haq Miyan[1], M. Mahbubur Rahman[1*]

[1]*Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh*

[2]*Faculty of Fisheries, Bangabadhu Sheikh Mujibur Rahman Agricultural University, Gazipur -1706, Bangladesh*

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Abstract

Studies were conducted to isolate and identify *Escherichia coli* from deteriorated rice and eggs as well as to evaluate antibiotic sensitivity pattern and screen some herbal extracts for their antibacterial activity against *E. coli*. A total of 10 from 52 isolates were identified by a series of morphological, physiological and biochemical tests. Eight antibiotics were tested by disc diffusion method where 90% of *E. coli* isolates showed resistance against sulphamethoxazole but found to be sensitive against gentamicin, ciprofloxacin and tetracycline. Erythromycin, streptomycin and cephradin showed 20% resistance in their sensitivity test results. However some medicinal plant extracts have been investigated during the study to assay their antibacterial activity against *E. coli* isolates so that diarrhea and dysentery associated with these bacteria can be treated by those fastidious herbs. Among 32 herbal extracts tested 12 extracts showed antibacterial activity against *E. coli*. Leaf extracts of *Temarindus indicus*, *Terminalia chebula, Citrus aurantifolia*, *Eugenia caryophyllus*, *Spondius pinnata* and bulb extracts of *Raphanus sativus* were able to exhibit antibacterial activity against all *Escherichia Coli* isolates. Thus medicinal herbs would be the better solution for the effective treatment of *E. coli* associated diarrhea and dysentery.

*Corresponding Author: M. Mahbubur Rahman ✉ mahbub_biotech@yahoo.com

Introduction

Escherichia coli has been associated with a number of diseases in human body as opportunistic pathogen. Among them diarrhea and dysentery are the most common which occur mostly due to food poisoning. Deteriorated or spoiled foods are the main causes of diarrhea and dysentery. The enterobacter *E. coli* are gram-negative, facultatively anaerobic bacteria that include a number of human pathogens i.e. *E. coli* O157:H7 and also a large number of spoilage organisms. These bacteria are widespread in nature in soil, on plant surfaces and in digestive tracts of animals and are therefore present in many foods (Doyle, 2007). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls. Sensitivity of *E. coli* to various commercial antibiotics varies from different types (serotypes) and sources (viz. UTI infection, poultry and deteriorated foods) of isolates. *In vitro* sensitivity pattern of *E. coli* to commercial antibiotics have been done earlier by some scientists as for probable solution of *E. coli* associated diseases. They reported that *E. coli* generally resistant to sulphamethaxazole and some other beta-lactam antibiotics while sensitive to ciprofloxacin, tetracycline and gentamicin (Ongsakul *et* al. 2009). Long use of antibiotics can lead to problem of bacterial resistance and release potential hazardous residues in the living environment. Thus the applications of antibiotics as a therapeutic agent for treatment of disease are now strongly discouraged in many developed countries. However, a large number of herbs are known to contain strong antibacterial activity that can be used to control different diseases. But, only a few scientific studies have yet been conducted in this field. Several countries started to use herbal compounds as an alternative of antibiotic treatment due to least side effects on human body. Considering the above facts, the present study was carried out to isolate and identify *E. Coli* from spoiled foods (rice and egg), to find out their antibiotic sensitivity pattern and to screen the inhibitory effect of available medicinal plant extracts for the isolates.

Materials and methods

Collection of sample

The samples were collected from spoiled rice and fried egg of two days stored in box (Table 1). A small part of egg and a single grain of rice was carefully taken with inoculating loops and streaked in nutrient agar plate. The culture plates were then incubated for 24 hours at 37°C. For the isolation and identification of *E. coli*, the enriched sample was cultured on selective medium Levine Eosin Methylene Blue (EMB) Agar and incubated at 37 °C for 24 hours.

Morphological characterization

The bacterial isolates were identified up to species level following the tests described in the Cown and Stell's Manual for the Identification of Medical Bacteria (Barrow and Feltham, 1993)

Physiological characterization

Physiological characterization of *E. coli* isolates were done under microscope to observe the shape, arrangement and presence of flagella. *E. coli* isolates were also allowed to grow in 4°C and 37°C.

Biochemical characterization

The *E. coli* isolates were characterized by a series of biochemical tests viz. Gram's test, catalase test, oxidase test, oxidative-fermentative (OF) test, indole test, methyl red test, Voges Proskauer, H_2S production test, acid production from glucose, gelatin hydrolysis test and motility test. *E. coli* isolates were also observed carefully under microscope for the presence of polar flagellum.

Colony counting

After isolation and characterization, *E. coli* isolates were diluted for colony counting (cfu) by serial dilution method on nutrient agar plate. Ten fold, hundred fold and thousand fold dilution were taken for colony

counting. Colony counting (Table 1) was done by spread plate method of John D. Buck and Robert C. Cleverdon (1960).

Table 1. *E. coli* isolates with their origin.

Isolates	Source	Days of isolation	Colony morphology	Colony color	CFU/gm
Ri_1	Rice	2nd day	Small, circular	White, moist and glistening growth	4.6-8.2×10⁶
Ri_2	Rice	2nd day	Small, circular	Slimy, white, somewhat translucent, raised growth	4.6-8.2×10⁶
Ri_3	Rice	3rd day	Small, circular	White, moist and glistening growth	7.2-11.6×10⁶
Ri_4	Rice	3rd day	Medium, circular	White, moist and glistening growth	7.2-11.6×10⁶
Ri_5	Rice	3rd day	Small, circular	Slimy, white, somewhat translucent, raised growth	7.2-11.6×10⁶
Eg_1	Egg	2nd day	Small, circular	Slimy, white, somewhat translucent, raised growth	5.5-9.8×10⁶
Eg_2	Egg	2nd day	Small, circular	White, moist and glistening growth	5.5-9.8×10⁶
Eg_3	Egg	2nd day	Small, circular	Slimy, white, somewhat translucent, raised growth	5.5-9.8×10⁶
Eg_4	Egg	3rd day	Medium, circular	Slimy, white, somewhat translucent, raised growth	9.8-16.7×10⁶
Eg_5	Egg	3rd day	Medium, circular	White, moist and glistening growth	9.8-16.7×10⁶

Antibiotic sensitivity test of E. coli isolates

Sensitivity of the *E. coli* isolates to different commercial antibiotics were determined by disc diffusion method as described by Rahman and Hossain (2010). Briefly, individual isolates were cultured into nutrient broth and incubated at 37°C for 24 hours. 50 µl of individual broth culture was dropped on the nutrient agar plate with micropipette. The broth on the plate was spread aseptically by a sterile 'L' shaped glass rod. Eight commercially prepared antibiotics discs *viz*., chloramphenicol (30 µg disc-'), erythromycin (10 µg disc-'), oxytencycline (30 µg disc-'), streptomycin (10 µg disc-'), sulphamethaxazole (15 µg disc-'), cefradine (30 µg disc-'), gentamicin (10 µg disc-'), ciprofloxacin (5 µg disc-'), cephalexin (5 µg disc-') were placed on the surface of the medium with sterile forceps and pressed gently to ensure good contact with the surface of the medium. The plates were then incubated at 37°C for 24 h. After incubation the organism was considered sensitive if there was zone of complete inhibition around the disc and resistant if there was no zone of inhibition. The diameter of the discs and the diameter of the zone of inhibitions were measured by measuring scale. The ratio between the diameters was calculated as described by Foysal *et*. al. (2011).

Antibacterial activity of herbal extracts to E. coli isolates

A total of 32 herbal extracts (Table 2) from 31 randomly selected herbs were used in this study to screen their antibacterial activity to the *E. coli* isolates. Most of the herbs were collected from a nursery of Adamdighi, Bogra and different parts of Sylhet district. The fresh parts of plants such as young leaves, burk, bulb, root, flower, rhizome or petiole were collected and washed several times with distilled water. The plant parts were cut into small pieces and paste was made by using mortar-pestle. Approximately 10µl of individual herb extract was inoculated onto the spread plate culture. The plate was then allowed to incubate at 37°C for overnight. After 12-24 h of incubation, the herb extract was noted for zone of inhibition for each

E. coli isolates. The diameter of the herbal extracts and the diameter of the zone of inhibition were measured by measuring scale. The ratio between the diameters was calculated.

Table 2. Plant parts used on the present study.

Local name	Botanical name	Parts of plant used
Durbagas	*Cynodon dactylon*	Leaf
Thankuny	*Centella asiatica*	Leaf
Helencha	*Alternamthera philoxeroides*	Leaf
Arjun	*Terminalia arjuna*	Leaf
Nim	*Azadirachta indica*	Leaf
Eucalyptus	*Eucalyptus camaldulensis*	Leaf
Ada	*Zingiber officinale*	Rhizome
Peaj	*Allium cepa*	Bulb
Rasun	*Allium sativum*	Bulb
Pan	*Piper betel*	Leaf
Dholkalmi	*Ipomoea fistulosa*	Leaf
Amrah	*Spondias pinnuata*	Leaf
Mehdi	*Lawsonia inermis*	Leaf
Kagaji lebu	*Citrus aurantifolia*	Fruit juice
Nayantara	*Catharanthus roseus*	Leaf
Nayantara	*Catharanthus roseus*	Flower
Haritaki	*Terminalia chebula*	Leaf
Tulshi	*Ocimum sanctum*	Leaf
Bel	*Aegle marmelos*	Leaf
Bashok	*Adhatoda vasica*	Leaf
Amloki	*Phyllanthus embelica*	Fruits
Labango	*Eugenia caryophyllus*	Leaf
Tentul	*Tamarindus indicus*	Leaf
Bahera	*Terminalia belerica*	Leaf
Jiga	*Lannea coromandelica*	Leaf
Golmarich	*Piper nigrum*	Leaf
Kadam	*Anthocephalus chinensis*	Leaf
Lajjabati	*Mimosa pudica*	Leaf
Pepe	*Carica papaya*	Leaf
Dhania	*Coriandram sativum*	Leaf
Nageshwar	*Mesua nagesarium*	Leaf
Kachu	*Colocasia exculenta*	Leaf

Results

Identification of the bacteria

A total of 52 isolates were taken from spoiled rice and egg sample. Among these, 10 isolates were confirmed as *E. coli* on the basis of a series of morphological, physiological and biochemical tests. The isolates were gram negative, rod shaped motile bacteria, able to produce acid from glucose and gave positive result in catalase test, oxidase test, methyl red test, indole test and found to be negative in Voges Proskauer test, H_2S production test and gelatin hydrolysis. The isolates were not able to grow at 4°C (Table 3).

Antibiotic sensitivity of the E. coli isolates

The *E. coli* isolates were found to vary in their antibiotic sensitivity pattern against eight commercial antimicrobial agents tested. All of the isolates showed sensitivity to two antibiotics viz. ciprofloxacin and gentamicin where most of the isolates found resistant to sulphamethaxazole (90%). Only one isolate (Eg1) was found to be sensitive to all eight antibiotics tested and only Ri5 isolate was found to be resistant to six antibiotics. Moreover, 20% isolates exhibit resistance to erythromycin, cefradine and streptomycin (Table 4).

Table 3. Common phenotypic properties of the *E. coli* isolates

Traits	Results
Gram stain	-
Shape	Rod
Motility	+
Oxidase	+
Polar flagella	+
Catalase	+
O-F test	Fermentative
Acid production in Glucose	+
Growth in 4°C	-
Growth in 37°C	+
Voges Proskauer	-
Indole production	+
H_2S production	-
Methyl red test	+
Gelatin hydrolysis	-

F = Fermentative; (-) Ve = Negative; (+) Ve = Positive

Antibacterial activity of herbal extracts to E. coli isolates

The *E. coli* isolates were found to be sensitive to 10 herbs out of 32 samples tested. The leaf extracts of *Temarindus indicus*, *Terminalia chebula*, *Eugenia caryophyllus* and *Spondius pinnata* inhibited the growth of all *E. coli* isolates whereas liquid juice extract of lemon (*Citrus aurantifolia*) and juice extracted from pulp of *Olium sativum* were also capable of inhibit the growth of *E. coli* isolates. Leaf extract of *Termenalia arjuna* and *Lawsonia inermis* also have the ability to inhibit *E. coli* isolates but in lower percentages. *Piper betle* and *Eukalyptus globulus* were also found to be quite effective only in some of *E. coli* isolates (Table 5).

Table 4. Antibiotic sensitivity pattern in percentage

Name of the Organism	Sensitivity pattern	% of isolated strain sensitive to various antibiotics							
		CIP	C	E	T	SXT	CE	GM	ST
Escherichia Coli	Resistant	0	10	20	10	90	20	0	20
	Less sensitive	10	20	20	30	10	30	20	30
	Moderately sensitive	20	30	40	30	0	40	40	30
	Highly sensitive	70	40	20	30	0	10	40	20

CIP: ciprofloxacin (5 µg disc-'), C: Chloramphenicol (30 µg disc-'), E: erythromycin (10 µg disc-'), T: tetracycline (30 µg disc-'), SXT: sulphamethaxazole (15 µg disc-'), CE: cefradine (30 µg disc-'), GM: gentamicin (10 µg disc-'), ST: streptomycin (10 µg disc-').

Table 5. List of medicinal herbs exhibited antibacterial effect on *E. coli* isolates. *(*Zone ratio denoted in scale of mm):

Medicinal herbs	Name of the *E. coli* isolates									
	Ri_1	Ri_2	Ri_3	Ri_4	Ri_5	Eg_1	Eg_2	Eg_3	Eg_4	Eg_5
Termenalia arjuna	1.2	1.7	1.5	1.7	2.0	1.4	2.1	2.0	2.0	1.9
Eukalyptus globulus	d	1.3	1.4	1.3	1.5	1.5	1.5	1.6	1.6	1.4
Olium sativum	2.4	2.7	2.8	2.5	2.9	2.6	2.8	3.0	2.7	2.6
Piper betle	1.5	1.8	1.9	d	1.9	1.7	1.8	2.0	d	2.2
Spondias pinnata	2.4	2.6	2.6	2.6	2.0	2.5	2.9	2.4	2.5	3.0
Lawsonia inermis	1.9	1.7	1.4	1.5	1.6	1.5	1.7	1.8	1.4	1.7
Citrus aurantifolia	2.1	2.4	2.7	2.5	2.8	2.4	2.3	2.5	2.7	2.9
Termanalia chebula	2.3	2.3	2.0	1.8	1.8	2.1	2.2	1.9	1.8	2.4
Eugenia caryophyllus	1.8	1.6	1.8	1.9	1.8	2.0	2.1	2.0	2.2	1.8
Tamarindus indicus	2.3	2.2	2.0	2.0	2.5	2.4	2.0	2.0	2.3	2.3

Discussion

E. coli has been considered as common bacteria associated with food spoilage since for many years and also directly involved for diarrheal disease throughout the world. The present study was conducted to identify *E. coli* from spoiled rice and egg, to assay antibiotic sensitivity pattern and to find out effective herbal extracts against *E. coli* isolates that might be prevent or cure *E. coli* associated disease such as diarrhea, urinary tract infection etc. In vitro antibiotic sensitivity of the *E. coli* isolates to eight commercial antibiotics viz. ciprofloxacin, chloramphenicol, erythromycin, tetracycline, sulphamethaxazole, cefradine, gentamicin and streptomycin were performed by disc diffusion method. In present study, all of the isolates were found as sensitive to only ciprofloxacin and gentamicin. 90% of isolates were found to be resistant to sulphamethaxazole and chloramphenicol followed by tetracycline (80%), erythromycin, cefradine and streptomycin (20%). Ongsakul M *et* al. (2009) reported that *E. coli* isolates were found to be sensitive to ampicillin, tetracycline, gentamicin and streptomycin. The reports correlate with our findings but with a quite high resistant pattern to streptomycin and tetracycline might be a sign of indication of increasing resistance of antibiotics to bacteria. *E. coli* that commonly associated with diarrhea and urinary tract infection exposed highly resistant to common antibiotics so the disease associated with *E. coli* is being very difficult to treat with those commercial third generation antibiotics. Meanwhile some local herbs and plant parts and citrus fruits posed excellent inhibitory activity against these bacteria. Leaf extracts of *Temarindus indicus*, *Terminalia chebula, Eugenia caryophyllus*, *Spondius pinnata,* liquid juice of *Citrus aurantifolia* and juice extracted from pulp of *Olium sativum* were capable of inhibit the growth of all *E. coli* isolates. Leaf extract of *Piper betle*, *Termenalia arjuna* and *Lawsonia inermis* were also found to be quite effective in some isolates. Bin Shan *et*. al (2007) stated antimicrobial activity of Clove (*Eugenia caryophyllus*) against pathogenic *E.* coli. Hasan M (2010) also found antibacterial activity of *Spondius pinnata* and *Temarindus indicus* against multi-drug resistant *E coli* from urinary tract infection during his B.Sc thesis in Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh. In present study, leaf extracts of *Ipomoea fistulosa* also found to be active against *E. coli* but its toxic effect upon on human body need to be further evaluated as a potential drug. Further research works are needed for purification of particular compounds responsible for antibacterial activity.

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