Samples were collected aseptically in clean, sterile bottles and immediately cultured on xylose lysine deoxycholate (XLD) agar overnight at 37 oC. Samples of yellow color were then cultured on plates containing Eosin Methylene Blue (EMB) agar to differentiate and select *E.coli* by incubating at 37oC (overnight). From EMB, subcultures of each sample were done on nutrient agar for biochemical tests.

2.2 Biochemical tests

Testing for indole production is important in the identification of enterobacteria. Most strains of *E. coli* breakdown the amino acid tryptophan with the release of indole. The test organism was cultured in a medium containing tryptophan. Indole production was detected by using Kovac‘s or Ehrlich‘s reagent, which contains 4 (p)-dimethylaminobenzaldehyde. This reagent reacts with the indole to produce a red- colored compound. The test organism was inoculated in a medium test tube containing 3 ml of sterile peptone water, and incubated at 35–37oC for up to 48 h. followed by testing for indole by addition of 0.5 ml of Kovac‘s reagent, shaken gently and examined for red color in the surface layer within 10 minutes (Vila ,1999).

2.3 Sero-typing

A drop of normal saline was placed on a clean microscope slide, inoculation from culture was added and a suspension was made, followed by addition of a drop of antisera.The slides were then rotated for 10 min and examined under the microscope to detect any clumping. Different reagents for different strains of *E.coli* were used that included: Enter pathogenic *E.coli* (EPEC), Entero invasive *E.coli* (EIEC), Entero Toxogenic *E.coli* (ETEC) and Entero homoraggic *E.coli* (VTEC). From each bacterial culture, four suspensions in four different slides were made for the four reagents, each reagent testing specific strains of *E.coli* (Sehgal, 1999).

2.4 Susceptibility of isolated bacteria to different antibiotics

In this study, sensitivity of the different isolates to a number of antibiotics that are commonly used in hospitals and community was studied using the standard disc diffusion technique. Mueller Hinton sensitivity testing agar medium was prepared and sterilized as instructed by the manufacturer and poured into sterile Petri dishes. The inoculums were prepared by emulsifying 3-5 colonies of the test organism in about 3-5 ml of sterile normal saline. A sterile cotton swab was dipped into this diluted culture; the swab was pressed against the side of the tube to remove excess fluid, and streaked across the medium in three directions by rotating the plate approximately 60º to ensure even distribution. (Williams and Ryan, 1998; Robins-Browne, 1987). A paper discs saturated with a known amount of the antibiotic were used. The antibiotic discs were carefully placed on the inoculated plates; each disc was lightly pressed down to ensure its contact with the agar. After overnight incubation at 37 ºC aerobically, the culture was examined for zones of inhibition of bacterial growth around the respective discs.

2.5 Interpretation of zone sizes

Using the interpretative chart, the zone sizes of each antibiotic was determined to classify the organisms as resistant, intermediate/moderately sensitive, sensitive (susceptible) (Robins-Browne, 1987).

3. Results

The total samples collected in the present study were 45, out of this, 30 were E.coli (showed metallic green on EMB) and 10 of them did not give positive result (metallic green) on EMB and the indole test (Table 1). Other remaining 5 samples didn't ferment the sugar on XLD (XLD media did not change to the yellow color) so they were rejected (Table 1).

All of the E. coli samples were subjected to sero-typing tests in order to classify those strains to: EHEC, EPEC, VTEC and EIEC. The results of sero-typing tests are summarized in Table 2 and Figures 4 and 5 shows the clumping of bacterial cells as a result of reaction with antisera.

Antibiotic sensitivity tests were done using the standard disk diffusion method. A measurement for zone of inhibition of bacterial growth was measured in millimeters (Table 3).

Table 1: Identification of stool samples (Culture media and biochemical testing)*

Sample number	*XLD*	*EMB*	*Indole test*
1	yellow	Metallic green	+ve
2	yellow	Metallic green	+ve
3	yellow	Metallic green	+ve
4	yellow	Metallic green	+ve
5	yellow	Metallic green	+ve
6	yellow	Metallic green	+ve
7	yellow	Metallic green	-ve
8	yellow	Metallic green	+ve
9	yellow	Metallic green	+ve
10	yellow	Metallic green	+ve
11	yellow	Metallic green	+ve
12	yellow	Metallic green	-ve (Suspected to be new strain)
13	yellow	Metallic green	+ve
14	yellow	Pink (unus ual case)	+ve
15	yellow	Metallic green	+ve
16	yellow	Metallic green	+ve
17	yellow	Pink (unus ual case)	+ve
18	yellow	Metallic green	+ve
19	yellow	Metallic green	+ve
20	yellow	Metallic green	+ve
21	yellow	Metallic green	+ve
22	yellow	Metallic green	+ve
23	yellow	Metallic green	+ve
24	yellow	Metallic green	+ve
25	yellow	Metallic green	+ve
26	yellow	Metallic green	+ve
27	yellow	Metallic green	+ve
28	yellow	Metallic green	-ve (Suspected to be new strain)
29	yellow	Metallic green	+ve
30	yellow	Metallic green	+ve

*XLD = Xylose Lysine Deoxycholate agar EMB= Eoin

Methylene Blue.
+ve= appearance of Red surface layer, -ve=No appearance of Red surface l ayer

Table 2: Identification of bacterial pathotypes using four different types of antisera

Sample Number	*EIEC*	*VTEC*	*EPEC*	*ETEC*
1	-ve	-ve	-ve	-ve
2	-ve	-ve	-ve	-ve
3	-ve	-ve	-ve	-ve
4	-ve	-ve	+ve	-ve
5	-ve	-ve	-ve	-ve
6	-ve	-ve	-ve	-ve
7	-ve	-ve	-ve	-ve
8	-ve	-ve	-ve	-ve
9	-ve	-ve	-ve	-ve
10	-ve	-ve	+ve	-ve
11	-ve	-ve	-ve	-ve
12	-ve	-ve	-ve	-ve
13	-ve	-ve	+ve	-ve
14	-ve	-ve	-ve	-ve
15	-ve	-ve	-ve	-ve
16	-ve	-ve	+ve	-ve
17	-ve	-ve	-ve	-ve
18	-ve	-ve	-ve	-ve
19	-ve	-ve	-ve	-ve
20	-ve	-ve	-ve	-ve
21	-ve	-ve	-ve	-ve
22	-ve	-ve	-ve	-ve
23	-ve	-ve	+ve	-ve
24	-ve	-ve	+ve	-ve
25	-ve	-ve	-ve	-ve
26	-ve	-ve	-ve	-ve
27	-ve	-ve	-ve	-ve
28	-ve	-ve	-ve	-ve
29	-ve	-ve	+ve	-ve
30	-ve	-ve	+ve	-ve

*EPEC=Enteropathogenic E.coli
EIEC= Entero invasive E.coli
ETEC= Entero Toxogenic E.coli
VTEC=Entero homoraggic E.coli
-ve = no clumping appears
+ve -= clumping appearance under microscope

Table 3: Inhibition zones for seven common used antibiotics against pathogenic E.coli

Samples containing pathogenic E.coli	Na	Ac	As	Co	E	C	AX
Sample No. 4	30	19	24	30	14	0	25
Sample No. 10	29	18	24	32	13	0	24
Sample No. 13	25	18	21	27	9	0	20
Sample No. 16	28	20	24	30	12	0	23
Sample No. 23	29	19	24	30	12	0	20
Sample No. 24	28	18	23	31	12	0	23
Sample No. 29	30	14	24	32	13	0	23
Sample No. 30	29	14	23	31	12	0	25

Na= Nalidixic Acid (30 mcg)
Ac= Amoxycillin / Clavulanic acid (30 mcg) As =Ampicillin / Sulbactam (10/10 mcg)
Co= Co-Trimoxazole (Trime thoprim/Sulphamethoxazole) (1.25/ 23.75 mcg) E= Erythromycin (15 mcg)
C= Chloramphenicol (30 mcg)
AX= Amoxicillin (25 mcg)
Inhibition zones in (mm)

Table 4: Resistance patterns for seven common used antibiotics against pathogenic E.coli*

Sample number containing pathogenic E.coli	Na	Ac	As	Co	E	C	AX
4	S	S	S	S	I	R	S
10	S	S	S	S	I	R	S
13	S	S	S	S	R	R	I
16	S	S	S	S	R	R	I
23	S	S	S	S	R	R	I
24	S	S	S	S	R	R	I
29	S	I	S	S	I	R	I
30	S	I	S	S	R	R	S

Na= Nalidixic Acid (30 mcg)
Ac=Amoxycillin / Clavulanic acid (30 mcg) As =Ampicillin / Sulbactam (10/10 cg)
Co =Co-Trimoxazole (Trime thoprim/Sulphame thoxazole) (1.25/ 23.75 mcg) E= Erythromycin (15 mcg)
C= Chloramphe nicol (30 mcg) AX= Amoxicillin (25 mcg)
S= Sensitive
I= Intermediate R= Resistant

Figure 1: Samples cultured on EMB media shown metallic green E.coli colonies

Figure 2: Testing samples for indole production

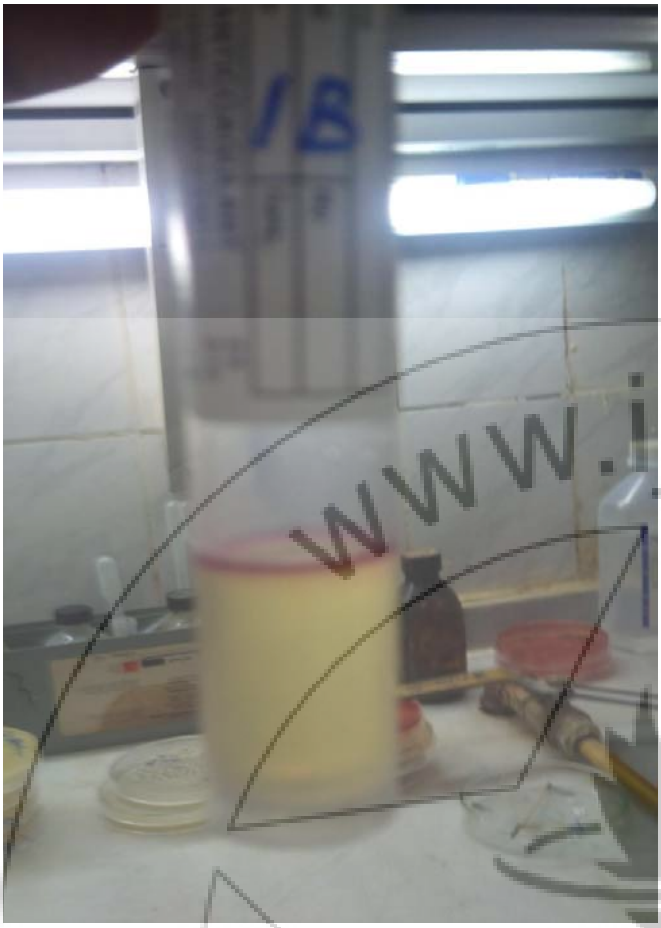

Figure 3: Positive indole sample (red surface layer)

Figure 4: Clumping of bacterial cells on slide as a result of reacting with antisera

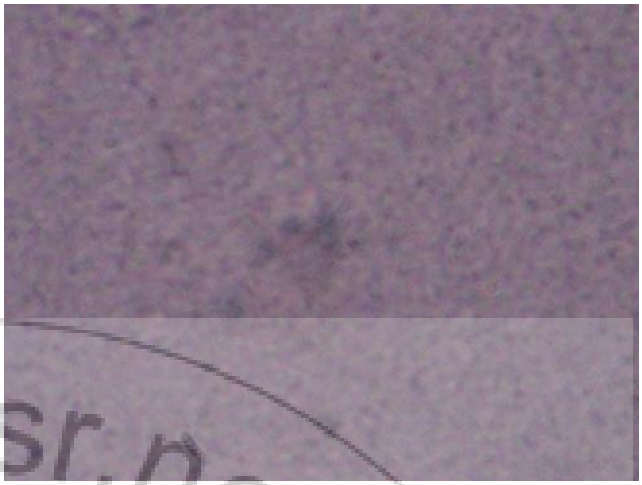

Figure 5: C lumping of bacterial cells under microscope as a result of reaction with antisera.

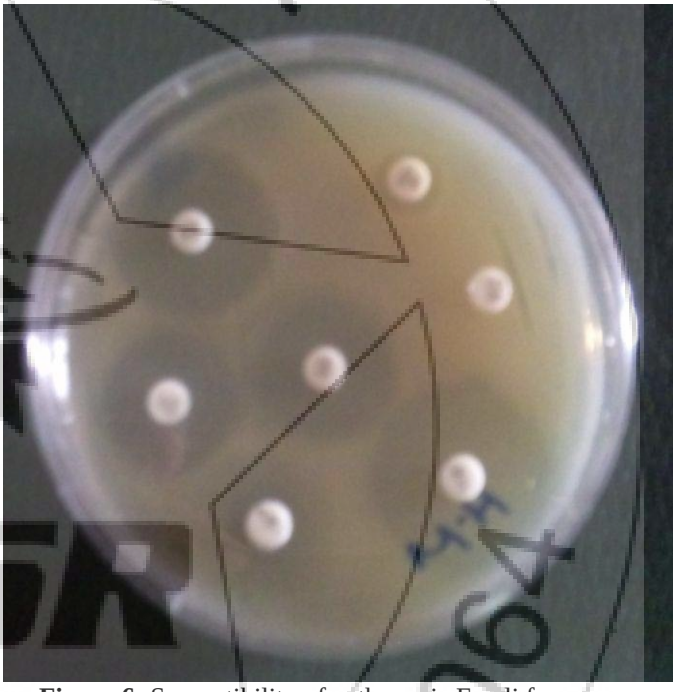

Figure 6: Susceptibility of pathogenic E.coli for seven common used Antibiotics

4. Discussion

The present study determined the resistance of E. coli isolated from stools of young children. The isolation and identification of bacteria were done on samples collected under strict sterile conditions to avoid contamination.

Antimicrobial resistance has become a major medical and public health problem. The main factor responsible for development and spread of bacterial resistance is injudicious use of antimicrobial agents, which has resulted in most gram positive and gram negative bacteria continuously developing resistance to the antimicrobials in regular use at different time periods (Urassa, 1997). The antimicrobial sensitivity is routinely performed to identify the best possible regimen for the treatment of bacterial infections. There are several reports from different parts of the world about antimicrobial sensitivity with varying results indicating the bacterial resistance to antimicrobials differs in different areas. In

Sudan, antimicrobial sensitivity tests were performed on four-hundred and ninety-seven bacterial isolates from patients with diarrhea, and enteropathogenic Escherichia coli. The results showed high resistance rates against the commonly used antimicrobial agents; ampicillin, amoxicillin, chloramphenicol, tetracycline, cotrimoxazole and nalidixic acid (Atif et al., 2000). Similarly, a study in Vietnam on 162 diarrheagenic E. coli isolates reported 77.2% resistance to chloramphenicol and 19.1% resistant to nalidixic acid (Trung et al., 2005).

The prevalence and antimicrobial susceptibility of diarrheagenic Escherichia coli in 346 children from Tanzania showed that diarrhea was due to enteropathogenic E. coli and there was high-level resistance to ampicillin, tetracycline, cotrimoxazole, and chloramphenicol but susceptible to quinolones (Vila et al.,1999). As mentioned earlier, the prevalence of resistance to commonly used antibiotics in groups of children from four developing countries; Peru, Belize, Zaire and Sudan (Vila et al. ,1999) showed that isolated microorganism were sensitive to ampicillin, tetracycline, cotrimoxazole, streptomycin and chloramphenicol. In the present study, all samples containing enteropathogenic E.coli were resistant to chloramphenicol, 62.5% of them were resistant to erythromycin, and intermediate sensitivity of 72.5% was observed for amoxicillin. The results of the present study were similar to those reported by Vila et al. (1999) and Atif et al. (2000). However, the sensitive of pathogenic Escherichia coli to nalidixic was different from that reported by Trung et al. (2005), where they found 19.1% of isolated pathogenic Escherichia coli res istant to nalidixic acid and in the present study no resistance was observed.

During informal discussion with children's mothers, we learnt that 90% of young children had used different classes of antibiotics during the last 6 months. We believe that use of these antibiotics might have led to of evolution of new resistant strains of bacterial pathogens. There was also strong correlation between hygiene, child activity and the infection with diarrhea. It is well known that indiscriminate use of some antibiotics, especially in young children leads to development of resistance to other classes of antibiotics apart from serious adverse effects associated with their use.

The susceptibility of enteropathogenic Escherichia coli for cotrimoxazole this study was high (100% effectiveness against bacteria under study). This result was contradictory to that reported in isolates from four different countries by Vila et al., (1999), where high level of resistance was observed for cotrimoxazole. The Ampicillin/Sulbactam combination was also very effective with 0% resistance. This wide spectrum antibiotic is most commonly used to treat diarrheal diseases.

Chloramphenicol failed to prevent the growth of enteropathogenic E. coli indicating that this drug is not suitable for use against these bacteria any more.

From the results of the present study, it is recommended that a routine program must be developed for regular sensitivity screening of commonly used antibiotics in the treatment of diarrhea. The regular determination of antibiotic sensitivity will help in reducing incidence of bacterial resistance and in prevention of adverse effects due to indiscriminate use of ineffective antibiotics. Furthermore, the use of highly effective antibiotics has a possible health risk, particularly for children under five. The routine screening of sensitivity will also help in selection of antibiotics with less adverse effects, especially in young children.

5. Conclusion

The enteropathogenic E. coli strains isolated from children under five years of age were found to be highly resistant to chloramphenicol, while amoxicillin showed a weak effect. Intermediate effect for amoxycillin/clavulanic acid and a very weak effect for erythromycin were observed. The E. coli strains were high sensitive to nalidixic acid, ampicillin/sulbactam and cotrimoxazole.

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