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ASSESSMENT OF FUNGAL FILTRATES EFFICIENCY AGAINST *ESCHERICHIA COLI* IN COMPARISON WITH COMMON ARTIFICIAL ANTIBIOTICS

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Abstract

The study aim to isolate, identify and characterize the *Escherichia coli* and look for their antibiotics resistance in children with diarrhea in najaf, the study includes 60 samples of stools gathered from patients children visiting Educational AL–Zahraa Hospital for the period beginning of November 2016 to mid-January of the same year, the age of these children were less than ten years, specimens were phenotypic assays, microscopically examined and diagnosed by biochemical tests, the highest bacterial pathogens isolated were *Escherichia coli*.

The sensitivity of isolates of *E. coli* were examined for 11 types of antibiotics, *E. coli* exhibite different pattern of resistance to different antibiotics, it is have highest resistance to penicillin (ampicillin and carbenicillin) and it is have higher resistance for ceftazidime and cefepime, while have moderate resistance for aztreonam. It have lowest resistant rate to imipenem, meropenem and ertapenem. Also the same isolates of *E. coli* were examined by the *Pleurotus ostreatus* fungi filterates, which appeare asignificant values in the inhibition of growth of *E. coli* in petri dish, which reach 8 cm in compare with antibiotics that used in the study.

Key words : *Escherichia colli*, artificial antibiotics, diarrhea, phenotypic assays.

Introduction

Diarrhea is a serious and widespread diseases in the world as it affects children under the age of 5 years to the disease and are concentrated injury in infants aged from six months to two years (Al-Kaby, 2000). Acute diarrheal diseases are an important health problem among children under five in developing countries (Garcia-Rodas *et al*., 2011). It has been reported that diarrheal diseases cause approximately 3 million deaths worldwide per year (Bentley and Meganathan, 1982).

The main cause of death in severe cases of diarrhea due to dehydration resulting from the loss of body fluids necessary. Because whatever is causing the diarrhea malnutrition. Therefore, diarrhea and malnutrition are among the main reasons for the occurrence of deaths in many countries (Ribeiro, 2000). The pathogen of many of them : such as bacterial like *Escherichia coli*, *Salmonella* spp, *Shigella* spp and *Campyiobacter* and Viral like Rota virus, Corona virus and Adeno virus as well as the etiology parasitic infections and the most important *Entamoeba histolytica* and *Giardia lamblia* and yeasts such as *Candida albicans* (Jawetz *et al*., 2004).

Come diarrhea as a result of the entry of pathogens to gastrocoele for children through the food and drinks and hands contaminated with those pathogens or as a result of turning some members of normal flora to etiology, acceptable to the increasing percentage of the normal limit due to a change in the intestinal environment as a result of eating certain drugs or injury the child in one of aetiology making it easier for these microorganism to events the disease (Pabst *et al*., 2003).

Among the bacterial pathogens *E. coli* plays an important role in causing diarrhea in children.

EPEC (Enteropathogenic *E. coli*) is an important category of diarrheagenic *E. coli*, which has been linked to infant diarrhea in developing world (Al-Hilali and Almohana, 2011). Five different pathotypes of

diarrheagenic *E. coli* are well recognized based on their patterns of gastrointestinal disease: enteropathogenic *E. coli* (EPEC), entero-toxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC) and enteroinvasive *E. coli* (EIEC) (Katia *et al*., 2007).

Escherichia coli is a Gram-negative, facultatively anaerobic, non sporing rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms) (Singleton, 1999). Cells are typically about 2.0 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm^3 (Facts about E. Coli, 2015; Yu *et al*., 2014; CDC NCEZID, 2012). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. (Vogt and Dippold, 2005). The harmless strains are part of the normal flora of the gut and can benefit their hosts by producing vitamin K2 (Bentley and Meganathan, 1982) and preventing colonization of the intestine with pathogenic bacteria (Hudault *et al*., 2001; Reid *et al*., 2001). *E . coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards (Russel and Jarvis, 2001).

E. coli and other facultative anaerobes constitute about 0.1% of gut flora (Eckburg *et al*., 2005) and 2% fecal–oral transmission is the major route through, which pathogenic strains of the bacterium cause disease (Kubitschek, 1990). Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for fecal contamination (Papp–Wallace *et al*., 2011; Ishii and Sadowsky, 2008). A growing body of research, though, has examined environmentally persistent *E. coli*, which can survive for extended periods outside of a host (Ribeiro, 2000). The bacterium can be grown and cultured easily and inexpensively in a laboratory setting and has been intensively investigated for over 60 years. *E. coli* is a chemoheterotroph, whose chemically defined medium must include a source of carbon and energy. Organic growth factors included in chemically defined medium used to grow *E. coli* includes glucose, ammonium phosphate, mono basic, sodium chloride, magnesium sulfate, potassium phosphate, dibasic, and water. The exact chemical composition is known for media that is considered chemically defined medium (Ribeiro, 2000). *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. Under favorable conditions, it takes only 20 minutes to reproduce (Russel and Jarvis, 2001).

Antibiotic treatment of common bacterial infections plays a crucial role in reducing morbidity and mortality due to these disease, however, over use and misuse of antibiotics in the treatment of diarrhea could lead to increased antibiotic resistance (Feng *et al*., 2002). In this context, a study was undertaken to isolate, identify and characterize the *E. coli* pathotypes and their antibiotic resistance in children less than 10 years with diarrhea in Najaf/Iraq, among these antibiotics were used includes:

Ampicillin is an antibiotic used to prevent and treat a number of bacterial infections (Reid *et al*., 2001). This includes respiratory tract infections, urinary tract infections, meningitis, salmonella infections and endocarditis. It may also be used to prevent group B streptococcal infection in newborns. It is used by mouth, by injection into a muscle, or intravenously (Tortora, 2010). Common side effects include rash, nausea, and diarrhea. It should not be used in people who are allergic to penicillin.

Ceftazidime is an antibiotic useful for the treatment of a number of bacterial infections. It is a third-generation cephalosporin.As a class, cephalosporins have activity against Gram-positive and Gram-negative bacteria. The balance of activity tips toward Gram-positive organisms for earlier generations; later generations of cephalosporins have more Gram-negative coverage (Sharma *et al*., 2013).

Colistin (polymyxin E) is a polymyxin antibiotic produced by certain strains of *Paenibacillus polymyxa* var. *colistinus*. Colistin is a mixture of cyclic polypeptides colistin A and B. Colistin is effective against most Gram-negative bacilli and is used as a polypeptide antibiotic (Koch-Weser *et al*., 1970).

Carbenicillin is a bactericidal antibiotic belonging to the carboxypenicillin subgroup of the penicillins.

It has Gram-negative coverage which includes *Pseudomonas aeruginosa*, but limited Gram-positive coverage. The carboxypenicillins are susceptible to degradation by beta-lactamase enzymes, although they are more resistant than ampicillin to degradation. Carbenicillin is also more stable at lower pH than ampicillin (Beringer, 2001).

Cefepime is a fourth-generation cephalosporin antibiotic. Cefepime has an extended spectrum of activity against Gram-positive and Gram-negative bacteria, with

greater activity against both types of organism than third-generation agents)Thompson, 2007).

Aztreonam(trade names Azactam injection, Cayston inhalation) is a monobactam antibiotic used primarily to treat infections caused by gram-negative bacteria. Aztreonam has strong activity against susceptible Gram-negative bacteria, including *Pseudomonas aeruginosa*. It has no useful activity against Gram-positive bacteria or anaerobes. It is known to be effective against a wide range of bacteria, including *Citrobacter*, *Enterobacter*, *E.coli*, *Haemophilus*, *Klebsiella*, *Proteus*, and *Serratia* species (Mosby's Drug Council, 2006).

Ertapenem has been designed to be effective against Gram-negative and Gram-positive bacteria.

It is not active against MRSA,ampicillin-resistant enterococci, *Pseudomonas aeruginosa*, or *Acinetobacter* species. Ertapenem also has clinically useful activity against anaerobic bacteria.

There are a few adverse effects of ertapenem like confusion and headache, which may worsen to convulsions and seizures the manufacturers cannot comment on its safety in pregnancy (Papp–Wallace *et al*., 2011).

Imipenem (Primaxin) is an intravenous β-lactam antibiotic. It was the first member of the carbapenem class of antibiotics. Carbapenems are highly resistant to the β-lactamase enzymes produced by many multiple drug-resistant Gram-negative bacteria (Hudault *et al*., 2001).

Meropenem : It is a β-lactam and belongs to the subgroup of carbapenem, similar to imipenem and ertapenem (Edwards *et al*., 1989). The spectrum of action includes many Gram-positive and Gram-negative bacteria (including *Pseudomonas*) and anaerobic bacteria.

The distinctive role of the fungus *P.o* in the fighting against many pathogens as well as improvement plant growth and productivity, as the fungus worked to reduce the incidence and severity of radicals disease caused by fungus Fusarium spp in tomato, eggplant, potato, pean, wheat and rice plants (Harman, 2000), the mechanisms that used by *T. harzianum* fungus in the fight against diseases were paratisim, enzymes secretion (Chitinase, Cellulase, Protease, β-1, 3gluconase), antagonisim, production of antibiotics (Trichodermol, Trichodermin, Pachybasi Gliotoxine, Emodin Chrysophancol), compotition and plant growth inducing (Harman *et al*., 2000).

Materials and Methods

Study was carried out at Educational Al-Zahraa hospital and microbiology laboratory, College of Science, University of Kufa in Najaf during 2016, Iraq. Children in the age group of less than 10 years, suffering from diarrhea and suspected *Escherichia coli*.

Sample collection

Stool specimens were collected from the children with diarrhea under 10 years of age over a period of approximately 3 months. The samples were collected in disposable sterilized seal-leak containers containing transport solution Carry Blair transport medium (is a semisolid medium recommended for use in the transportation and preservation of clinical specimens , primarily stool and rectal swabs).

Sample analysis

The specimen was cultured according to standard method. In order to evaluate the role of *E.coli* small amount of each samples were cultured initially on MacConky Agar and incubated for 24 hrs. at (35-37^{0}C) ,the remainder stool from each samples inoculated into Selinite Broth for detection of pathogenic bacteria and incubated tubes for 24hrs. at (35–37^{0}C). On second day read results macroscobically for *E.coli* on MacConky Agar, which appears pink in color, the specimens were checked microscopically by Gram stain which appear negative Bacilli and diagnosed by biochemical tests (IMVIC), which incubated for 24hrs. at (35-37^{0}C) and read the results in third day, transport specimen from Selinite Broth to XLD and incubated 24 hrs. at (35-37^{0}C) for detection the presence of other pathogenic bacteria such as (Salmonella, Shigella).

On third day, the biochemical tests (MIVIC) performed for the specimens isolated from XLD and incubated for 24hrs. at(35-37^{0}C), read the results in the fourth day, which appear dry yellow colonies and compared with the result of the first (IMVIC).

On fourh day, done the final steps of diagnosis (biochemical reaction) on the samples from both XLD and MacConky Agar, compared the results, which includes :

A- Kliger Iron Agar , red in color which became acid bottom/acid slant give gas but no H_2S.

B- Urea, yellow in color which give negative result because *E.coli* lack Ureas enzyme.

C- Indol, palle yellow in color which give positive result and produced red ring after incubated one day and added dropes from indicater for it.

D- Monitol Salt Broth that blue in color became yellow with mortility.

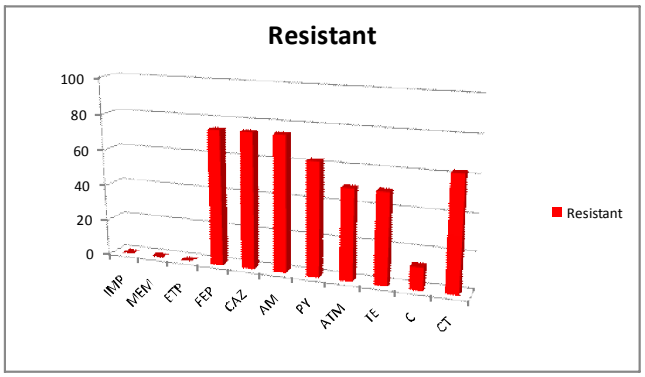


Fig. 1 : Antibiotics resistance of *E. coli* isolates by disk diffusion method.

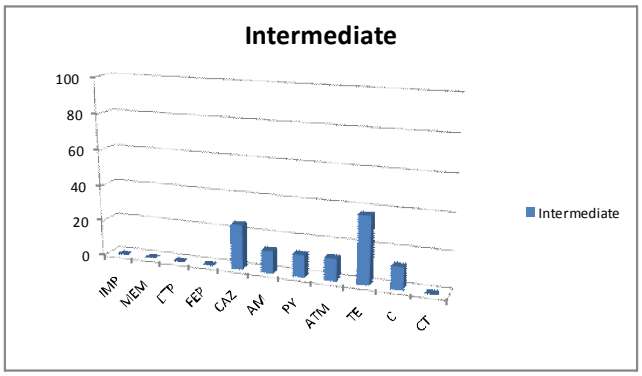


Fig. 2 : Antibiotics Intemediate of *E. coli* isolates by disk diffusion method.

Table 1 : Biochemical tests of *E.coli* Isolates.

E. coli (11 isolates)	Test
-	Gram stain
-	Catalase
-	Oxidase
-	H_2S
+	Indole
+	Methyl red
-	Vogasproskauer
-	Citrate utilization
-	Urease
+	Motility
+	Lactose
+	Acid from glucose
+	Sucrose

Note:- result of final steps of biochemical tests read after incubation for 24 hrs. at(35-37^{0}C).

Transport and activate of isolated specimens

The isolated specimens of *E. coli* placed in Nutrient Broth in order to preserve the microorganism (*E. coli*) from decay for certain period were transported into Microbiological Laboratory, College of Science, University of Kufa and activate the bacteria (10 isolates)

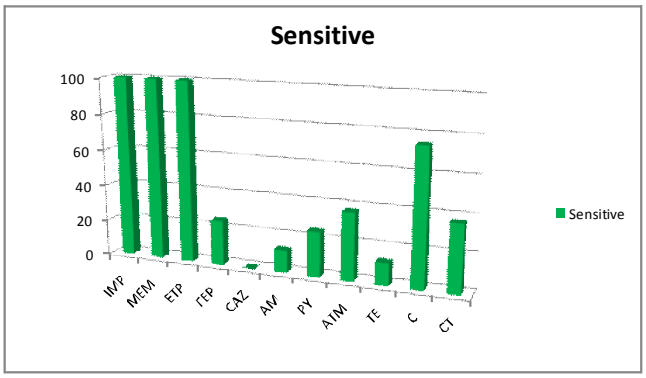


Fig. 3 : Antibiotics Sensitive of *E. coli* isolates by disk diffusion method.

by cultured on Brain heart Agar (37mg per 1000ml; used 9.25mg per 250ml), incubated for 24 hrs. at (35-37^{0}C).

Antibiotic sensitivity assay

Antibiotic sensitivity assay was performed by using 11 types of antibiotic discs includes : Cefapime, Tetracycline , Aztreonam, Ampicillin, Colistin Sulphate, Ceftazidim, Carbenicillin, Chloramphenicol 30, Ertapenem, Imipenem, Meropenem after cultured *E. coli* on Muller Hilton Agar (mg per 1000ml; used 9.5mg per 250ml) and read the results after incubation of 24 hrs. at (35-37^{0}C).

Addition of fungi filterates

To the petridish that swapped with *E.coli* and with some of antibiotics which used in this study (fungi filterate prepared by Dr. Nihad H.) in order to inhibit the growth of *E.coli.*

Antimicrobial susceptibility testing of β-lactam resistant E. coli isolates was performed on Mueller-Hinton agar plates by using (Kirby-Bauer) disk diffusion method against antibiotic listed in table 1. The cultures were incubated at 37°C for 18 hrs. under aerobic conditions and bacterial growth inhibition zones diameter were measured and interpreted in accordance with the Clinical and laboratory Standards Institute (CLSI) guidelines (CLSI, 2010). *E. coli* TOP-10 was used as the reference strain for antibiotic susceptibility testing.

Results

Antibiotic susceptibility tests of *E.coli* Isolates

As determined by disk-diffusion method, all *E. coli* isolates exhibited different pattern of resistance to different antibiotic agents (fig. 1), demonstrating highest resistance to penicillins (ampicillin and carbenicillin) with rate of resistance of (75% and 62.5%) isolates, respectively.

Resistance to other drug classes varied among the isolates. For cephalosporin antibiotics, a higher resistance

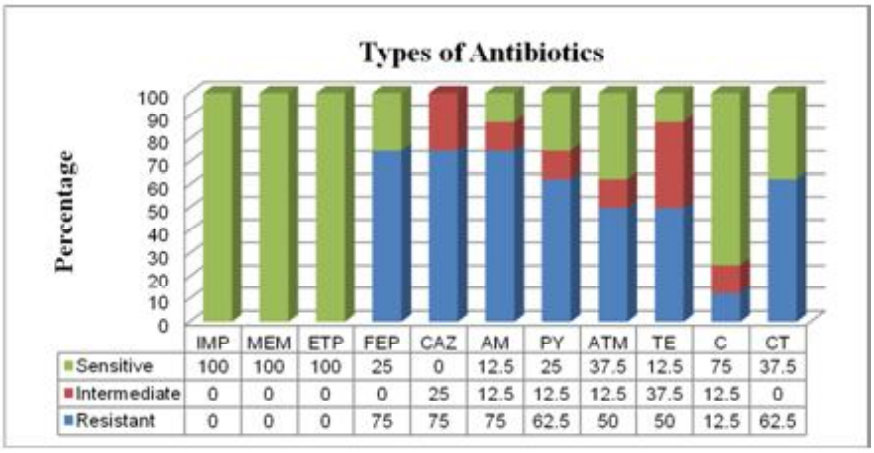


Fig. 4 : Total antibiotics susceptibility profile of *E. coli* isolates by disk diffusion method (IMP, Imipenem; MEM, Meropenem; ETP, Ertapenem; FEP, Cefepime; CAZ, Ceftazidime; AM, Ampicillin; PY, Carbenicillin; ATM, Aztreonam; TE, Tetracycline; C, Chloramphenicol; CT, Colistin sulphate).

Table 2 : The antibiotics used in this study.

Antibiotic class	Agent used	Symbol	Content	Origin
Penems	Imipenem	IMP	10	BioMaxima
	Meropenem	MEM	10	BioMaxima
	Ertapenem	ETP	10	BioMaxima
Cephems	Cefepime	FEP	30	BioMaxima
	Ceftazidime	CAZ	30	BioMaxima
Penicillins	Ampicillin	AM	10	Bioanalyse
	Carbenicillin	PY	100	Bioanalyse
Monobactams	Aztreonam	ATM	30	Bioanalyse
Tetracyclines	Tetracycline	TE	30	BioMaxima
Phenicols	Chloramphenicol	C	30	Bioanalyse
Polymyxins	Colistin sulphate	CT	10	BioMaxima

Table 3 : Effect of addition of fungi filterates on inhibition of groth of E.coli on petri dish.

Antibiotic	Inhibition Diameter (cm)						
	C	ETP	AM	CAZ	MEM	ATM	IP
E.coli +Antibiotic	2.30	3.8	0	0.8	2.9	1.7	2.2
Fungi filtrate	8						

was also detected with (75%) of isolates being resistant to ceftazidime and cefepime. The results also revealed that were moderate resistant rates (50%) isolates for aztreonam of monobactam's antibiotics.

For the carbapenem antibiotics, imipenem, meropenem and ertapenem displayed the lowest resistant rate (0%) isolates.

Percentages of resistance of isolates to the remaining antibiotics were as follows : (50%) for tetracycline, chloramphenicol (12.5%), Colsitin sulphate (62.5%). Results revealed that some tested isolates were resistant to a minimum 3 classes of antibiotics, hence these isolates were considered to be multidrug resistant.

The important results that obtained (fig. 1) were the resistance of *E.coli* against the IMP, MEM, ETP, so it must pay attention that this antibiotics were useless therapy as an important components in manufacturing midicines , also this antibiotics had ahighest sensitivity against *E.coli* bacteria (figs. 2, 3, 4).

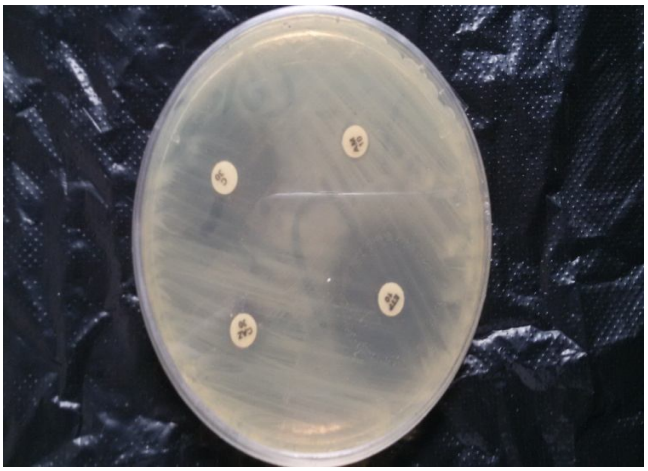
Addition of fungi filterates to petridish cotain *E. coli*

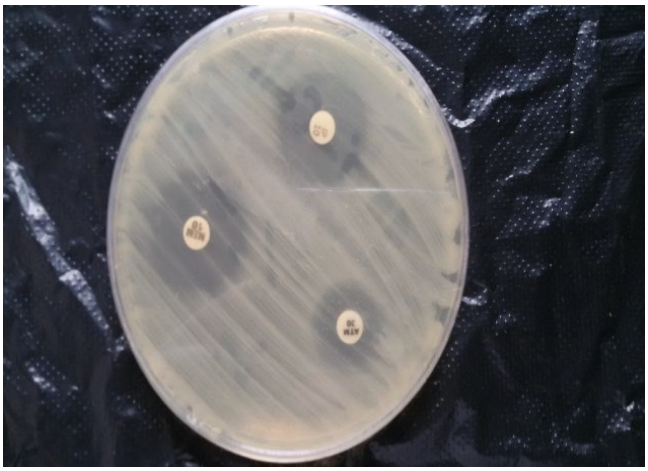
The results appearse that the antibiotics ETP, MEM, C, IP, ATM, CAZ gave different inhibition diameters against *E.coli*, which reach (3.8, 2.9, 2.3, 2.2, 1.7, 0.8) cm, respectively, while AM antibiotic not effect on growth of this bacteria, in another hand asignificant result was obtained that the fungus filtrates gave ahigher inhibition zone in compare with above treatment (*E.coli* +Antibiotic) which also compare with control treatment which reach 8 cm, so it mean that fungi filtrates gave asignificant control against *E.coli* (table 3, images 1, 2, 3, 4).

Conclusion

The result and discussion of our study's research leads us to that "Ability to manufacture a new therapy for *E. coli* control" and we arrived to these conclusions:

1. Diarrhea is a serious and widespread diseases in the world as it affects children under the age of 5 years in Al-Najaf province.





Images 1,2 : Antibiotics + *E.coli*.

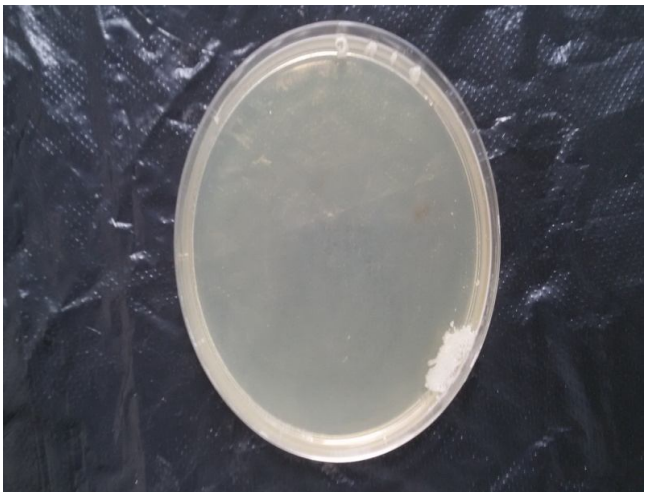


Image 3 : Fungi Filtrates + *E. coli*.

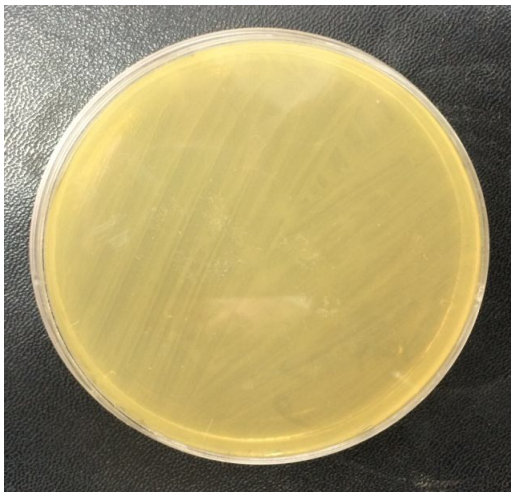


Image 4 : Control (*E. coli*).

2. There were some of antibiotics were useless for *E.coli* remediation.
3. Utilizing fungi filtrates for *E.coli* treatment with or with out the antibiotic that used in our study.

Recommendation

1. Provide hospital medical pharmicies about the useless antibiotics and publish official informations (Appendix) about it.
2. Establishing educational programs about retesting aprevious therapies.
3. Advise people to following the healthy ways to avoid diarrhea daily.
4. Application of lifestyle modification that should be availability ahealthy environment.
5. Further studies should be done to explore other factors associated with diarrhea.
6. Addition anew pharmaceutical technology for manufacturing anew generation of therapy.

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