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Research Article

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Screening and Characterization of Waterborne *E. coli* from Children with Diarrheal Infections and Evaluation of the Bactericidal Efficacy of Plant Extracts and Nanoparticles Against Pathogenic Isolates.

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ABSTRACT

Bacterial infections including diarrhea are most common among children. Current study involved the screening of pathogenic *E. coli* from children with diarrheal infections. Stool samples were collected from pediatric departments of public hospitals aseptically using autoclaved swabs containing Cary Blair transporting medium. Six diarrheal stool samples were spread on EMB agar media. All samples showed the presence of *E. coli* except one sample. Isolated bacterial colonies were streaked on nutrient agar plates for pure culture. Pathogenicity test was done using blood agar medium. Two strains exhibited beta hemolysis. Antibacterial activity of different biological agents such as antibiotics, nanoparticles and plant extract were also studied. Seven antibiotics solutions (Amoxicillin, ceftriaxone, chloramphenicol, ciprofloxacin, oxytetracycline, Doxycycline, Azomax) and one antibiotic disc (Meronem) were applied to check the resistance/sensitivity of bacterial strains against them by well diffusion method and disk diffusion method respectively. The zones of inhibition of these antibiotics or antibiotic susceptibility for all these strains were different. Four silver nanoparticles of *Cassia fistula*, *Eucalyptus*, *Azadiracta indica*, *calotropis proceri* and four plant extracts (*Cassia fistula*, *Euclyptus*, *Azadiracta indica*, *calotropis proceri*) were applied against isolated *E. coli* strains. The zone of inhibition of these nanoparticles and plant extracts for all these strains were different. Strain 2 showed maximum sensitivity against Meronem with 12mm Zone of inhibition while same strain was resistant against chloramphenicol, Doxycycline and oxytetracyclin. Bacterial isolates were resistant against plant extracts but showed susceptibility against nanoparticles. It can be concluded from current study that *E. coli* is associated with diarrheal infections among children. The prevalence of this infection can be prevented via hygienic conditions and proper treatment using different antibacterial agents.

Keywords: Diarrhea, Antibiotic susceptibility, Nanoparticles, plant extracts

INTRODUCTION

Diarrhea is the sudden beginning of excessive bowel movements brought on either directly or indirectly by microbial infections. It is the second largest reason for death on a global scale. There are 3 million fatalities each year from diarrhea. This virus affects mostly young children in poor nations. This infection is 1000 times more common in the US. Food poisoning (whether caused by microbes or some other reason), bacterial infections due to *E. coli, Shigella, Salmonella, Campylobacter*, *Yersinia*, viral infections due to Rotavirus, and protozoan infections due to *Entamoeba, Giardia lamblia* are the causes of diarrhea. Scientist Theodor Escherich, a German physician in year 1885, gave the bacillus *Escherichia coli* its name after first isolating it from the feces of infants with diarrhea. Based on biotyping and serotyping, it is a diverse group of bacteria. It causes diarrheal infections in both people and animals. *E. coli* considered as Gram-negative bacteria. It measures 1-4 x 0.4–0.7 microns. It can be found alone or in pairs. Because they have flagella of the peritrichate kind, *E. coli* are mobile. There are very few non-motile strains. *E. coli* are not spore-forming or non acid-fast bacteria. *E. coli* functions as facultative anaerobe and aerobic bacteria. It grows best at 37°C, and its range lies between 10 and 40°C. It produces big, thick, wet, opaque, grey colonies on plain nutrition agar media. It yields lactose fermenters that are a vivid pink color on MacConkey media. Greenish colonies are produced on EMB agar media (Ahmed *et al.*, 2016). *E. coli* strains are categorized into six major types that cause diarrhea, which include; Enterohemorrhagic, Enterotoxigenic, Enter invasive, Enteropathogenic and Enteroaggregative Diffuse-adherent All pathotypes show different pathogenesis, and have different virulence properties. (Pitout *et al.*, 2005). India's cholera-like diarrhea was

first identified as being caused by this pathotype in the late 1960s. Similar to *vibrio cholerae* they produce toxins that affect the mucosal cells and cause watery, copious diarrhea. They adhere to but do not enter the small intestine mucosa. Little inflammation and no obvious histological alterations are present [1]. Shiga toxin-producing *E. coli* (STEC) is another name used for this pathotype because their toxins are closely related to Shiga toxin and they have a cytotoxic impact on Vero cells, that's why also named Vero cytotoxin *E. coli* (VTEC). Konowachuk was the first to describe it in 1977. One specific strain of *E. coli* (O15:H7) belonging to this pathotype was identified to be the responsible agent in two outbreaks of the recognizable bloody diarrheal illness in 1983 (Fischer *et al.,* 1996). Its virulence factors are Vero cytotoxin, Intimin and EHEC plasmid. *In vivo* invasion of intestinal epithelial cells is a capability of enteroinvasive *E. coli*. *Shigella* spp. and EIEC strains have close biochemical, genetic, and pathogenetic ties. Just like *Shigella* spp., EIEC strains are typically non-motile, lactose-negative, with some exceptions such as lesser acid resistance and inability to make Shiga toxin (Jafari *et al.,* 2008). The majority of the time, they induce watery diarrhea, and on rare occasions, they also cause dysentery syndrome (Al-Hilli *et al.,* 2010) The capability to enter host tissues is encoded through the acquisition of the invasive plasmid (pINV). Penetration in epithelium cells, dissolution of the endocytic vacuole, multiplication inside the cell, directed movement through the cytoplasm, and movement into neighboring cells of epithelium are all aspects of its pathophysiology (Majeed & Aljanaby, 2019). In the poor world, newborn diarrhea has been associated with an important group of diarrhea-causing *E. coli* called EPEC. The first three years of Human life are the time when most EPEC infections happen. EPEC infections exhibit pronounced seasonality and peak during the warm seasons. A four-stage approach was proposed by a scientist Knutton in 1998; Localized adherence, Signal transduction: Intimate adherence: Pedestal formation (Hebbelstrup *et al*., 2018). It is named from the way that they gather together on Hep-2 cells resembling stacked bricks. After ETEC, it is the second typical reason for traveler's diarrhea in both industrialised and developing nations (Khajanchi *et al.,* 2010). This pathotype is well-acknowledged as the root cause of both endemic and epidemic diarrhea. It has persistent diarrhea as a side effect. Its pathogenesis involves adhesins and aggregative adherence fimbriae (AAF) colonizing the intestinal mucosa. Mucoid biofilms form. Different enterotoxins, cytotoxins, and mucosal inflammation are produced (Vila *et al.,* 2003). HeLa and HEp-2 cells have a widespread adhesion pattern due to the heterogeneous group. It is linked to recurrent urinary tract infections and permanent watery diarrhea among children in both developed and developing nations. There are two categories of virulence factors: Afa/Dr adhesins: which have diverse intestinal roles and induce urinary tract infections. and Diffuse adherence-related adhesins: a possible contributor to infantile diarrhea. (Mansan *et al*., 2013)

E. coli related diarrheal illnesses are treated with antibiotics. However, the abuse of antibiotics has resulted in an increase in bacterial and microbial strain resistance to antibiotics. The main causes of the rise in antibiotic resistance are mutation or gene transfer. Nanoparticles are also used for treatment and their mode of action differs from that of antibiotic resistance because they are directly connected to the bacterial cell wall without passing through it. As a result, it is believed that nanoparticles may reduce the development of antibiotic resistance in bacterial strains (Wang and Hu, 2017). To prevent a variety of bacterial infections, silver nanoparticles were used because of their anti-microbial and anti-bacterial properties (Franci *et al.,* 2015). Nanoparticles release silver ions, increasing their antimicrobial activity (Priyadarshini *et al.,* 2019). Due to their effectiveness, low cost, and ease of production, these properties make these nanoparticles excellent for use in the medical industry and health care products. The use of plants and herbs as an alternative for antibacterial activity has come under study from the scientific and pharmaceutical worlds due to the rise in diseases that are resistant to antibiotics (Bruna *et al.,* 2021)

MATERIALS AND METHODOLOGY

Area of Study: The area of study for the collection of Diarrheal samples was the pediatric department of Fatima Memorial Hospital (FMH) Lahore. It has a purpose-built campus located in Shadman, in the heart of Lahore.

Sample collection: Six Diarrheal stool samples were collected from the pediatric department of Fatima Memorial Hospital Lahore (FMH) using autoclaved swabs containing cary blair transporting medium. Samples were brought to Government College University Lahore. These Diarrheal samples were stored at 4℃ for the least possible bacterial activity and to run the samples**: Isolation of bacteria from diarrheal stool samples:** The isolation of bacteria from samples of diarrhea was done using EMB as a medium. *E. coli* was isolated using EMB as a selective medium. 3.5g of EMB agar was prepared by dissolving it in 50ml of distilled water. The media were then autoclaved for 20 minutes at 121℃ under 15 Ib of pressure. To prevent contamination, the medium was autoclaved and then transferred into sterilized petri plates following aseptic conditions. To allow the media to solidify, these plates were left to stand for five to ten minutes. These EMB agar plates were spread with the culture stick-collected samples of diarrheal stools. To check contamination, these plates kept in incubator overnight at temp 37°C. The *E. coli* bacterial colonies were observed.

Preparing Culture plates: The basic medium for isolating pure cultures was nutrient agar. By mixing

6.5g of broth and 8.5g of agar in 500ml of distilled water, the nutrient agar media were prepared. The media were then autoclaved for 20 minutes at 121^{0}C under 15 Ib of pressure. To prevent contamination, the medium was autoclaved and transferred into sterilized petri plates following aseptic conditions. To allow the media to solidify, these plates were left to stand for five to ten minutes. To check for contamination, these plates were left in the incubator for the night.

Isolation of pure culture: To prepare a pure culture, a single isolated bacterial colony was taken from an established bacterial colony on EMB agar plates and streaked onto solidified autoclaved nutrient agar medium, and kept in an incubator at temp 37°C for time 24 hrs (Figure 1).

Glycerol stock preparation: Stocks of glycerol were made to preserve a few different bacterial strains. Bacterial cultures were incubated overnight in an incubator at 37^{0}C in 50ml of broth medium. The 200L of sterilized glycerol stock mixed with 800L of culture broth in the autoclaved eppendorfs were placed under laminar air flow. Each bacterial strain had two sets, and after vortexing for ten seconds, glycerol stocks were chilled at 20^{0}C.

Pathogenicity test: An enriched medium made of blood agar was utilized to isolate fastidious bacteria. Blood in the medium provides complex nutrients and supplements needed by pathogens, which prevents the growth of *Neisseria spp*. and *Haemophilus* spp. 500ml of distilled water was used to dissolve 8.5g of agar and 6.5g of nutrient broth. The medium for this test was autoclaved. After autoclaving, the medium was cooled to 45°C, and 3ml of non-coagulated blood was added. Blood was added at this necessary temperature to prevent it from turning into chocolate agar, which promotes the growth of *Heamophilus* spp and *Neisseria* spp by causing the hemolysis of red blood cells when blood agar is heated, giving it a chocolate color. Blood agar was mixed, and then added to sterile petri plates, which were subsequently streaked with isolated cultures in laminar air flow and incubated at 37^{0}C. Growth appears to indicate positive outcomes. While greenish zones suggested alpha hemolysis and Gamma hemolysis showed no hemolysis, clear ones indicated ß hemolysis.

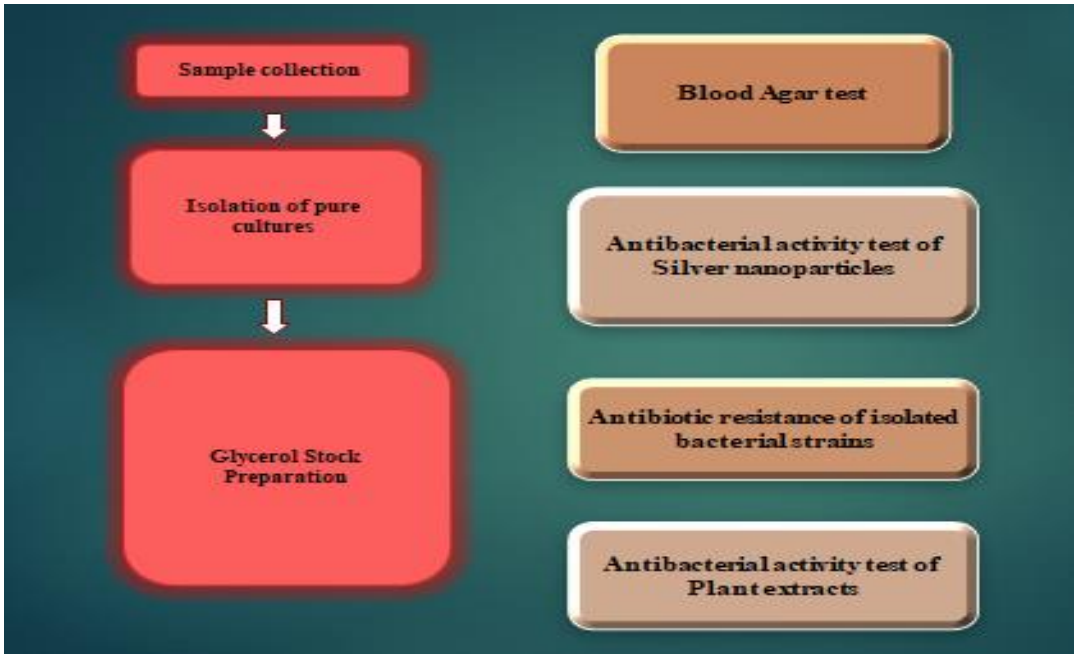

Figure 1. Schematic presentation of Methodology

Antibacterial activity test against antibiotics: Nutrient agar made by mixing 6.5g nutrient broth and 8.5g agar in 500ml of water. The autoclaved medium poured to six Petri plates under aseptic conditions and solidified. Following solidification, a sterile glass spreader was used to apply an isolated bacterial culture to the media. Using the well diffusion method, seven antibiotic solutions; amoxicillin, ceftriaxone, chloramphenicol, ciprofloxacin, oxytetracycline, doxycycline, and azomax were applied to nutrient agar plates. Using sterile forceps, one Meronem antibiotic disc was inserted into nutrient agar plates. The plates were then incubated overnight in an incubator set at 37°C. The zone of inhibition, or clear zone, that forms around antibiotics determines the sensitivity of isolated strains. With the aid of a measuring scale, the zone of inhibition was measured. Bacterial resistance to a particular antibiotic was visible in the growth around antibiotic solutions or antibiotic discs (Figure 2).

Antibacterial activity test of Nanoparticles (Green synthesis): For determining the antibacterial activity of various nanoparticles, the well diffusion method was used. 10g of the nanoparticles were dissolved in

100ml of deionized water to create the solution. Four different types of silver nanoparticles (SNPs) of *Calotropis procera*, *Eucalyptus*, Neem (*Azadirachta indica*), and *Cassia fistula* were used. In a flask filled with 1000ml of distilled water, 17g of agar and 13g of nutrition broth were dissolved to prepare nutrient agar. The medium was autoclaved at 121℃ for 20 minutes at 15 Ib pressure to sterilize it. After adding medium into each petri plates, four wells were made in each plate then bacterial culture was disseminated equally throughout the plate using a spreader. Separately, 100 µl of nanoparticle solution was poured into wells. Petri plates with lids were incubated at 37℃ for the entire night. Following incubation, a zone of inhibition around the wells revealed an isolate's sensitivity to a particular nano particle.

Antibacterial activity test of plant extract: Different plant extracts' antibacterial activity was evaluated using the well diffusion method. The plant extract solution was created by combining 20g of powder with 200ml of deionized water. There were four different kinds of plant extracts used: *Calotropis procera*, *Eucalyptus*, Neem (*Azadirachta indica*), and *Cassia fistula*. In a flask filled with 1000ml of distilled water, 17g of agar and 13g of nutrition broth were dissolved to create nutrient agar. The medium was autoclaved at 121℃ for 20 minutes at 15 Ib pressure. The medium was added in four Petri plates, after solidification four wells were made in each petri plate and then the bacterial culture was dispersed equally throughout the plate using a spreader.100µl of plant extract solution were placed into wells. Petri plates with lids were incubated at 37℃ for the entire night. A zone of inhibition around the wells after incubation showed the isolate's sensitivity to a particular extract

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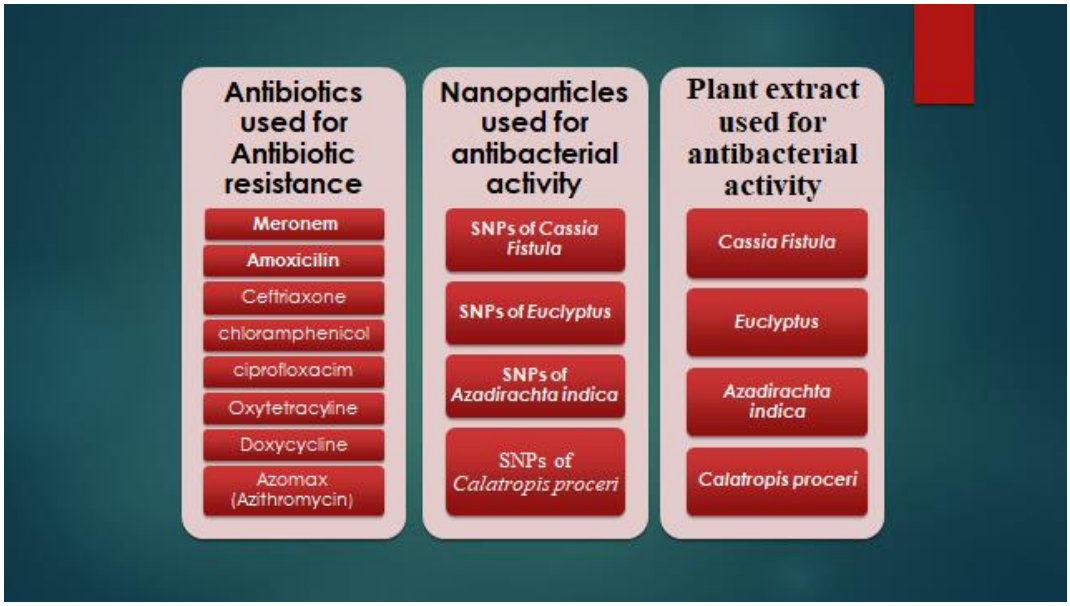

Figure 2. Schematic presentation of antibacterial assays

RESULTS

Five out of six samples showed the presence of *E. coli* by exhibiting greenish colonies on EMB agar medium. A total of five (Table 1 and Figure 1). Pure culture of isolated *E. coli* colonies showed white color with smooth texture and spherical shape (Table 2 and Figures 3 & 4).

Blood agar test of isolated strains: Strains 1 and 2 showed beta hemolysis while remaining three strains showed gamma hemolysis (Table 3). Results showed that strains 1 and 2 were pathogenic and associated with diarrheal infections (Figure 5).

Antibiotic resistance/sensitivity of isolated bacterial strains: Zone of inhibitions of strain 1 against antibiotics Meronem, Amoxicilin, Ceftriaxone, Chloramphenicol, Ciprofloxacim, Oxytetracycline, Doxycycline, Azomaz were 12.25mm, 2.9mm, 4.75mm, 7.5mm, 9.5mm, 6.9, 4.9, 6.9 respectively (Figures 6 & 10) and zone of inhibition of strain 2 against antibiotics Meronem, Amoxicilin, Ceftriaxone, Chloramphenicol, Ciprofloxacim, Oxytetracycline, Doxycycline, Azomax were 12.75mm, 0mm, 4.1mm, 0mm, 9.8mm, 0mm, 0mm, 6.5mm respectively (Table 4).

Antibacterial test of Nano particles: Zone of inhibition of strain 1 against SNps of following plant extracts; *Cassia fistula*, *Euclyptus*, *Azadiracta indica*, *Calatropis procera* were 5.5mm, 7, 5.5mm, 2.75mm respectively (Figures 7 & 9). Zone of inhibition of strain 2 against SNps following plant extracts; *Cassia fistula*, *Euclyptus*, *Azadiracta indica*, *Calatropis procera* were 3.5mm, 3.8mm, 3.4mm, 4.6mm respectively (Table 5).

Figure 3. Initial screening of E. coli on EMB agar

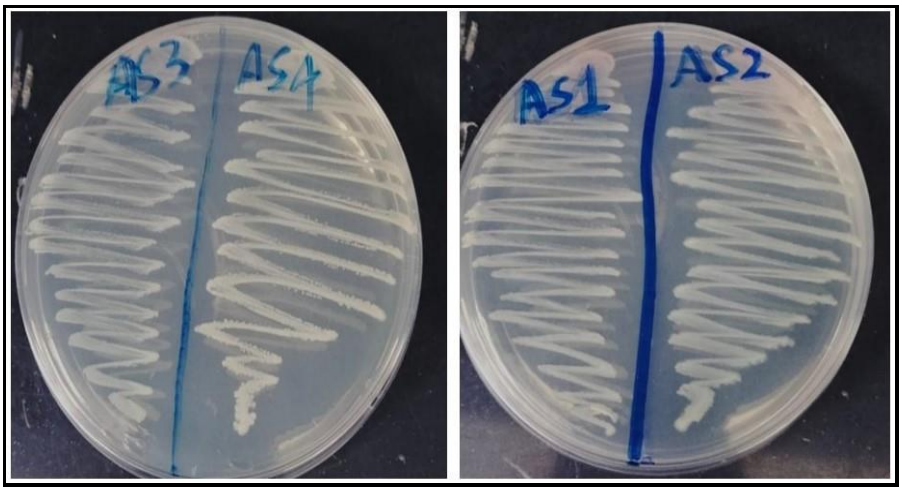

Figure 4: Streaked plates of isolated colonies

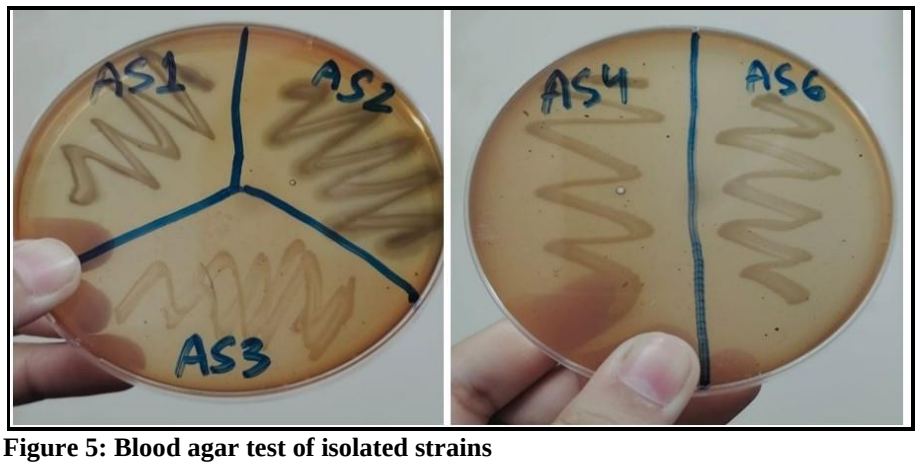

Figure 5: Blood agar test of isolated strains

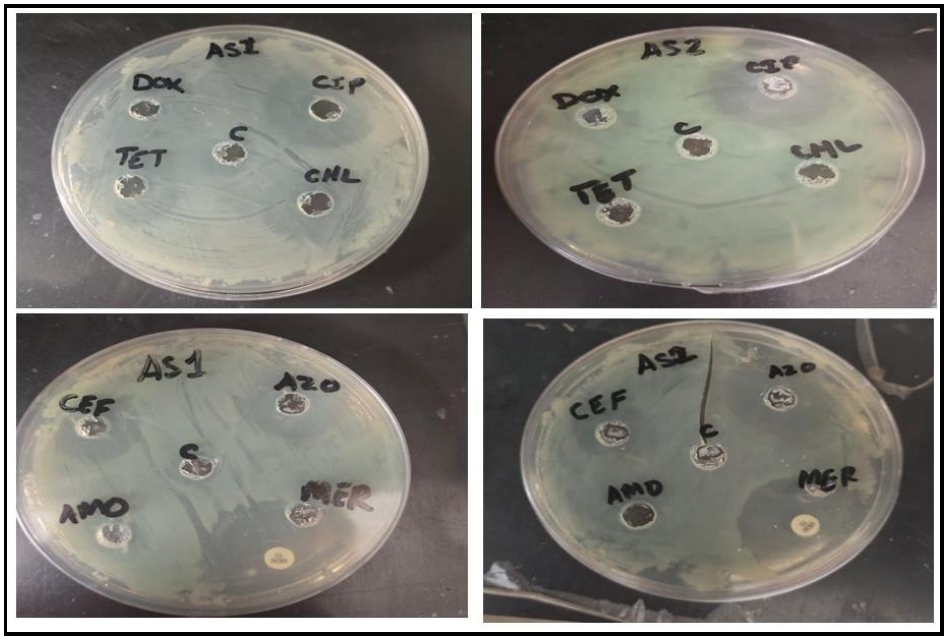

Figure 6: Antibiotic resistance of isolated bacterial strains

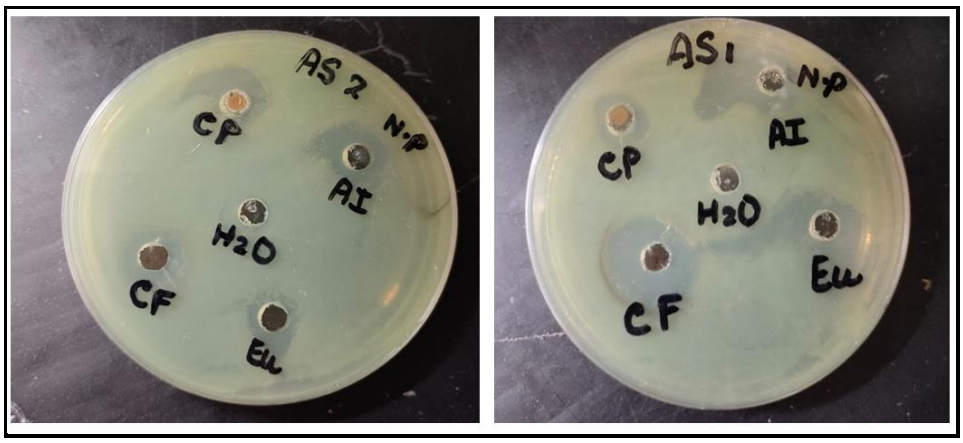

Figure 7. Antibacterial test of Nanoparticles

Antibacterial test of Plant extracts: Both strain 1 and 2 showed great resistance against the following plant extracts; *Cassia fistula*, *Euclyptus*, *Azadiracta indica*, *Calatropis procera* by exhibiting no zone of inhibition (Table 6 and Figure 8).

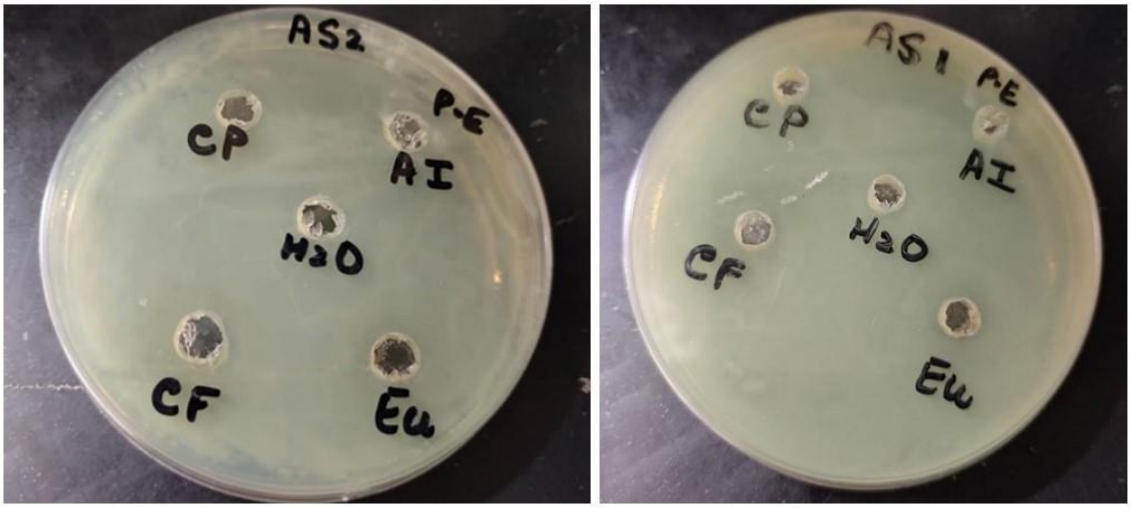

Figure 8: Antibacterial test of plant extracts

Table 1: Initial screening of E. coli on EMB agar medium

Sample	Results
Sample 1	Present
Sample 2	Present
Sample 3	Present
Sample 4	Present
Sample 5	Absent
Sample 6	Present

Table 2: Morphological Characterization of E. coli Isolates from stool samples on Nutrient agar Medium

Sample Name	**Strain**	**Streaking Type**	**Colony Characteristics**
AS1	Strain1	Continuous	Large, thick, greyish-white color, smooth texture, spherical shape
AS2	Strain2	Continuous	Opaque, smooth texture, Low convex, moist.
AS3	Strain3	Continuous	white color, smooth texture, Opaque
AS4	Strain4	Continuous	Opaque, Thick, large colonies, moist.
AS6	Strain5	Continuous	white color, smooth texture, Circular.

Table 3: Blood agar test of isolated strains

Strains	**Pathogenicity**	**Hemolysis**
Strain 1	+ve	Beta
Strain 2	+ve	Beta
Strain 3	-ve	Gamma
Strain 4	-ve	Gamma
Strain 5	-ve	Gamma

-ve (negative)
+ve (positive)

Table 4: Antibiotic Susceptibility Profile of E. coli isolates as Zone of Inhibition ± S.E

Antibiotics	**Zone of inhibition (mm)**	
	Strain 1 (AS1)	Strain 2 (AS2)
Meronem	12.25 ± 0.6	12.75 ± 0.1
Amoxicillin	2.9 ± 0.2	R
Ceftriaxone	4.9 ± 0.1	4.11 ± 0.3
Chloramphenicol	7.5 ± 0.2	R
Ciprofloxacim	9.5 ± 0.2	9.8 ± 0.3
Oxytetracycline	6.9± 0.3	R
Doxycycline	4.75± 0.1	R
Azomax	6.9 ± 0.2	6.5 ± 0.3

S.E = Standard Error

Table 5: Antibacterial test of Nanoparticles ± S.E

SNps of Plant extract	**Zone of inhibition (mm)**	
	Strain 1 (AS1)	Strain 2 (AS2)
Cassia Fistula	5.5 ± 0.4	3.5 ± 0.3
Eucalyptus	7.8 ± 0.5	3.8 ± 0.3
Azadiracta indica	5.5±0.2	3.4 ± 0.1
Calotropis proceri	2.7 ± 0.1	4.6 ± 0.2

Table 6. Zone of Inhibition (mm) for Antibacterial Activity of Plant Extracts against E. coli extract

Plant extract	**Zone of inhibition (mm)**	
	Strain 1 (AS1)	**Strain 2 (AS2)**
Cassia Fistula	R	R
Euclyptus	R	R
Azadiracta indica	R	R
Calotropis proceri	R	R

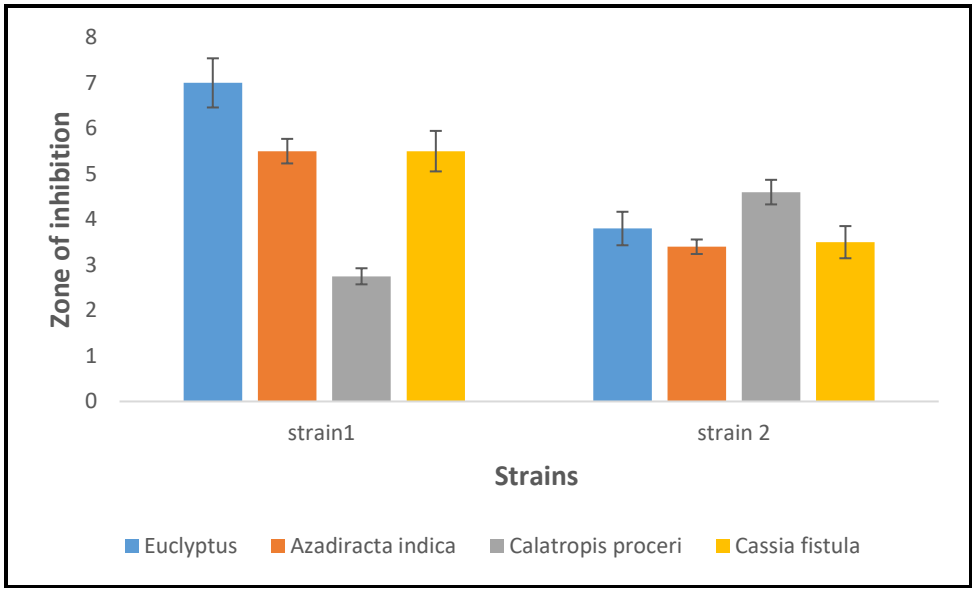

Figure 9. Antibacterial test of Nanoparticles ± S.E value

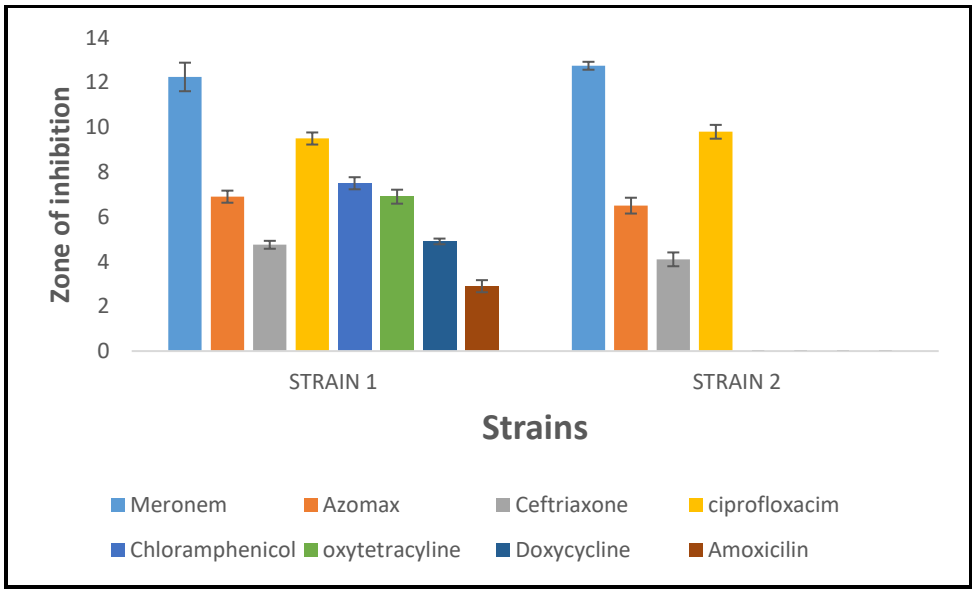

Figure 10. Antibacterial test of antibiotics ± S.E

DISCUSSION

As the years went by, it became clear that a variety of bacterial species can cause diarrhea. The distribution of diarrheal infections also expanded, making it more difficult in laboratories to identify these bacterial species. According to the latest research, diarrhea-causing *E. coli* (DEC) is one of the main causes of diarrheal infections. This study aims to gain an accurate understanding of infection control methods as well as the causes, risk factors, and transmission of infection.

The result of the current study against meronem antibiotic revealed that both strains of *E. coli* showed great zone of inhibition against this antibiotic and the results were supported by the past studies as meronem was considered a good antibacterial agent because of its efficacy and showed less resistance against *E. coli* species. Against azomax, ceftriaxone, ciprofloxacim, both strains of *E. coli* showed susceptibility. Strain 1 showed susceptibility against these antibiotics (Amoxicillin, chloramphenicol, oxytetracycline, Doxycycline.) but strain 2 showed resistance against Amoxicillin, chloramphenicol, oxytetracycline, and Doxycycline in the present study while the results of past findings showed almost similar results against *E. coli.* In past studies EPEC resistance to antibiotics is a growing problem. During the last 2 decades, extended-spectrum β-lactamases (ESBLs) found in Gram-negative bacilli have emerged as a significant mechanism of resistance to oxyimino-cephalosporin antibiotics. (Senthamarai *et al.*, 2014). The results of current study showed that antibiotics showed almost similar antibacterial activity because p- value was greater than 0.05. there was no significant difference in antibacterial activity of these antibiotics.

The results of the current study showed that nanoparticles of silver metal synthesized from different plant extracts showed almost similar antibacterial activity because p-value was greater than 0.05. There was no significant difference in antibacterial activity of the SNPs of *Eucalyptus*, *Calotropis procera*, *Azadirachta indica* and *Cassia fistula*. The results of current study showed the maximum zone of inhibition was 8.5mm against strain 1 and no resistance was shown by strain 1 against SNPs of plant extracts gives the best bactericidal effect (Hari and Bose, 2021). The m aximum zone of inhibition of strain 2 was 4.25 mm against SNPs of any plant extracts in the current study while the past findings also had similar results that green synthesized SNPs had good antibacterial activity against *E. coli* (Ahmed *et al*., 2016).

The result of current study exhibited that aqueous plant extract of *Eucalyptus*, *Calotropis procera*, *Azadirachta indica* and *Cassia fistula* showed resistance for both strains of *E. coli.* In past studies, results showed that aqueous extracts of *Azadirachta indica* did not show any good antibacterial activity against different pathogenic bacteria including *E. coli* While methanolic extracts show good antibacterial activity at different concentrations. (Francine *et al.*, 2015).The results of the current study confirmed the presence of *E. coli* in stool samples of diarrheal patients by biochemical characterization using specific media; EMB agar media.

CONCLUSION

The investigation concluded that *E. coli* is a major cause of pediatric diarrheal illnesses. Different antibiotics were employed to treat diarrheal infections, but because they were misused or overused to treat infections, bacteria became resistant to them. It is recommended that various biological agents be utilized as antibacterial agents to whom *E. coli* may not have acquired resistance, such as plant extracts and nanoparticles.

AUTHORS' CONFLICT

There is no conflict of interest among authors. All authors approved final draft of manuscript.

AUTHORS' CONTRIBUTIONS

Conceptualization and Editing: Nazish Mazhar Ali and Sara Hayee. Supervision: Nazish Mazhar Ali and Samreen Riaz. Investigation: Nazish Mazhar Ali and Muhammad Ahsan Raza Methodology: Ayesha Shahid and Hamna Junaid. Statistical analysis: Ayesha Shahid and Hamna Junaid.

REFERENCE

Ahmed, S., Saifullah, Ahmed, M., Swami, B.L. and Ikram, S., 2016. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. *J. Radiat. Res. Appl. Sci.,* 9:1-7.

Khajanchi BK, Fadl AA, Borchardt MA, Berg RL, Horneman AJ, Stemper ME, Joseph SW, Moyer NP, Sha J, Chopra AK. Distribution of virulence factors and molecular fingerprinting of Aeromonas species isolates from water and clinical samples: suggestive evidence of water-to-human transmission. Appl Environ Microbiol. 2010 Apr;76(7):2313-25.

Ikram, S. (2015). Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. Journal of Radiation Research and Applied Sciences, 30(1), e7.

Al-Hilli, Z. B. (2010). Dissemination of β-lactamases in Escherichia coli and Klebsiella spp. isolated from Merjan teaching hospital in Hilla city. College of Science, University of Kufa.

Bouzari, S., Oloomi, M., Aslani, M., & Jafari, A. (2010). Phenotypic and Genotypic Characterization of Enteropathogenic Escherichia coli (EPEC) strains in Tehran, Iran. Iranian Journal of Microbiology, 2(1), 3-7.

Bruna, T., Maldonado-Bravo, F., Jara, P., & Caro, N. (2021). Silver nanoparticles and their antibacterial applications. International journal of molecular sciences, 22(13), 7202.

Senthamarai, S., Sivasankari, S., Anitha, C., Somasunder, V., Kumudhavathi, M. S., Amshavathani, S. K., & Venugopal, V. (2014). Resistance pattern of Pseudomonas aeruginosa in a tertiary care hospital of Kanchipuram, Tamilnadu, India. Journal of clinical and diagnostic research: JCDR, 8(5), DC30.

Fischer, S. A., Harris, A. A., Segreti, J., Goodman, L. J., Langkop, C., Swartz, M., & Trenholme, G. M. (1996). Screening for Escherichia coli O157: H7 in Illinois. Clinical Microbiology and Infection, 1(3), 175-178.

Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G., & Galdiero, M. (2015). Silver nanoparticles as potential antibacterial agents. Molecules, 20(5), 8856-8874.

Majeed, H. T., & Aljanaby, A. A. J. (2019). Antibiotic susceptibility patterns and prevalence of some extended spectrum beta-lactamases genes in gram-negative bacteria isolated from patients infected with urinary tract infections in Al-Najaf City, Iraq. Avicenna journal of medical biotechnology, 11(2), 192.

Hari, A. and Bose, V.G., (2021). Synthesis of Silver Nanoparticles: Characterization and Antimicrobial Activity of Synergistic Herbal Formulation Derived from *Cassia fistula* and *Manikara zapota* Plants. *Gorteria J*., 34:1-10.

Jafari, F., Shokrzadeh, L., Hamidian, M., Salmanzadeh-Ahrabi, S., & Zali, M. R. (2008). Acute diarrhea due to enteropathogenic bacteria in patients at hospitals in Tehran. Japanese journal of infectious diseases, 61(4), 269-273.

Mansan-Almeida, R., Pereira, A. L., & Giugliano, L. G. (2013). Diffusely adherent Escherichia coli

strains isolated from children and adults constitute two different populations. BMC microbiology, 13, 1-14.

Pitout, J. D., Nordmann, P., Laupland, K. B., & Poirel, L. (2005). Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) in the community. Journal of antimicrobial chemotherapy, 56(1), 52-59.

Priyadarshini, S., Sulava, S., Bhol, R., & Jena, S. (2019). Green synthesis of silver nanoparticles using Azadirachta indica and Ocimum sanctum leaf extract. Current Science, 117(8), 1300-1307.

Trabulsi, L. R., Keller, R., & Gomes, T. A. T. (2002). 10.321/eid0805. Typical and Atypical Enteropathogenic Escherichia coli. Emerging infectious diseases, 8(5), 508.

Vila, J., Ruiz, J., Gallardo, F., Vargas, M., Soler, L., Figueras, M. J., & Gascon, J. (2003). Aeromonas spp. and traveler's diarrhea: clinical features and antimicrobial resistance. Emerging infectious diseases, 9(5), 552.

Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: present situation and prospects for the future. International journal of nanomedicine, 1227-1249.