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ISOLATION AND MOLECULAR IDENTIFICATION OF BACTERIA BY 16SRNA AND POLYMERASE CHAIN REACTION IN DIFFERENT WATER SAMPLES

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ABSTRACT: Anibiogram data of all the 10 isolates that grew on EMB agar and identified by biochemical tests were also cheked for antibiotic susceptibility and antibiotic resistance using antibiotics.All these isolates were confirmed upto genus level using IMViC and other biochemical test such as urease, catalase, H_2S production and sugar fermentation.Gentamycin, streptomycin, oflaxacin, norfloxacin and amoxicillin. Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating coliform bacteriainfection *in vivo*. Testing for antibiotic sensitivity is often done by the Kirby-Bauer method. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, is seen around the disc indicating poor growth. One isolate WMF1 was identified using molecular (16sr RNA) as E. coli *Escherichia fergusonii*.Genomic DNA was isolated using kit from xcelris for detection of *Lac Z* as the β-galactosidase gene and *uidA* beta-D-glucuronidase of*Escherichia coli*and detection of two genes from WMF 1 showed the presence of two genes. Lac Z corresponding to 500 bp and uidA gene corresponding to 147 bp confirming the presence of substrate utilizing genes in E. coli WMF1.

Key words: 16s RNA, PCR, LacZ gene, Antibiotic susceptibility

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INTRODUCTION

Transposon mutagenesis and the generation and analysis of gene fusions have been widely used in the study of gene organization and expression of a variety of organisms (Andrei 2007. Transposon mutagenesis allows the mutation, identification, and isolation of genes contained within large genetic targets, including prokaryotic, while gene fusions allow the analysis and manipulation of the expression and regulation of genes whose functions are unknown or difficult to assay (Neemann et al 2004; Abdul et al 2005; Abid et al 2005; Aihara 1997). In gene fusions, the control sequences of a gene of interest are placed in front of the coding sequences of a 'reporter' gene whose product can be readily assayed. Thus, expression of the gene can be monitored by measuring the reporter gene product, and genetic and environmental factors that affect the expression can be determined and manipulated. The Escherichia coli 3- galactosidase structural gene, lacZ, has often been employed as the reporter gene in the construction of gene fusions. ,B-Galactosidase is easily and quantitatively assayed, is amenable to genetic manipulations and is active in a variety of organisms and when contained within hybrid proteins (Alhussain et al 2005; Alabaster et al 1980; Aldridge et al 1978; Aljaro García et al 2005). Employing lacZ, two types of gene fusions can be made. Transcriptional fusions are generated using sequences that contain the lacZ gene including its translational initiation signal; expression of a transcription fusion results in the production of wild-type, B-galactosidase (Alonso et al 1994). In a translational fusion, the translational initiation signal and the amino-terminal coding sequences of a gene are linked 5' directly to the lacZ coding sequences; expression of a translational fusion results in the production of a chimeric protein with 3-galactosidase activity.

Both transcriptional and translational fusions are useful for studying prokaryotic gene expression (Bernhard 2004). Translational fusions also have a number of additional applications. They can be used to study gene expression in eukaryotic systems, because translation of the prokaryotic reporter gene is placed under the control of eukaryotic translational control sequences (Bej et al 1990; 1996; Bernhard et al 2000). They also can be used to determine the site of translational initiation of a gene and to analyze its control. Furthermore, a hybrid protein produced from a translational fusion can be useful for studying properties of the original protein encoded by the target gene (Biswas et al 1994). Transposable elements that contain lacZ coding sequences combine the techniques of transposon mutagenesis and the generation of lac gene fusions; such elements allow the random insertion of lacZ into a wide variety of DNA sequences (Bloch 1997; Cabello 1997). LacZ transposons that generate transcriptional gene fusions, or translational gene fusions have been constructed previously, and used to determine the location and the transcriptional orientation of genes and to analyze their expression. In this paper we describe the construction and use of a new Tn3-lacZ element, Tn3-HoHol. This transposon can be used in the generation of both transcriptional and translational lac fusions with plasmid DNA sequences, and these fusions are stable, as Tn3-HoHol cannot self-transpose.

MATERIALS AND METHODS

IMVic Test

Indole production test

Tryptophan, an essential amino acid, is oxidized by some bacteria by the enzyme tryptophanase resulting in the formation of indole, pyruvic acid and ammonia. The test is performed by inoculating a bacterium into tryptone broth; the indole produced during the reaction is detected by adding Kovac's reagent (dimethylamino benzaldehyde) (make HI-MEDIA), which produces a cherry red color. Cool the broth to 55^{o}C and then pour into four sterile test tubes.Inoculate three tubes with culture, and keep third tube as an un inoculated. Comparative control.Incubate the tubes at 35^{o}C for 48 hrs. After 48 hrs of incubation, add 1ml of Kovac's reagent to each tube including control.Shake the tubes gently after intervals for 10-15 minutes.Allow the tubes to stand to permit the reagent to come to top.

Methyl-red and Voges-proskauer tests

The methyl-red (MR) and the Voges-Proskauer (V-P) tests are used to differentiate two major types of facultatively anaerobic enteric bacteria that produce large amounts of acid and those that produce the neutral product acetoin as end product. Both these are performed simultaneously because they are physiologically related and are performed on the same medium MR-VP broth. Opposite results are usually obtained for the methyl-red and Voges-Proskauer tests, i.e. MR+, VP- or MR-, VP+. In these if an organism produces large amounts of organic acids: formic, acetic, and lactic and succinic (end products) from glucose, the medium will remain red (a positive test) after the addition of methyl red a P^{H} indicator (i.e. P^{H} remaining below 4.4). In other organisms, methyl red will turn yellow (a negative test) due to the elevation of the P^{H} above 6.0 because of the enzymatic conversion of the organic acids (produced during the glucose fermentation) to non-acidic end products such as ethanol and acetone.

Methodology

Preparation of MRVP broth (pH 6.9), Sterilize by autoclaving at 15lb pressure for 15 minute After autoclaving cool to 50^{o}C and then pour into four sterile test tubes. Inoculate three test tubes with culture and keep one tube as un inoculated. Comparative control.Incubate all four tubes at 35^{o}C for 48 hours.Add 5 drops of methyl red indicator to two tubes.

Citrate utilization test

Citrate test is used to differentiate among enteric bacteria on the basis of their ability to utilize/ferment citrate as the sole carbon source. The utilization of citrate depends on the presence of an enzyme citrase produced by the organism, which breaks down the citrate to oxaloacetatic acid and acetic acid. Inoculating the microorganisms into an organic synthetic medium, Simmon's citrate agar, where sodium cirate is the only source of carbon and energy, performs the cirate test. Bromothymol blue is used as an indicator. When the citric acid is metabolized, the CO_2 generated combines with sodium and water to form sodium carbonate an alkaline product, which changes the color of the indicator from green to blue and this constitutes a positive test:

Methodology

Weigh 3.4 Gms of Simmon's citrate agar (P^{H} 6.9) media and dissolve in 100ml of distilled water and sterilize and autoclaving at 15 lb pressure for 15 minutes.Cool the medium and pour into four sterile test tubes, and allow them to solidify in a slanting position.Inoculate three Simmon's citrate agar slants with culture by means of a stab-and-streak inoculation. The fourth tube is kept as auninoculated comparative control.Incubate all the tubes at 37^{o}C for 48 hours.

Other Biochemical tests: (Urease, Catalase, H_2S production)

Prepare media as mentioned above.Mix well and sterilize the media by filtration. Do not autoclave or heat the medium. Dispense in sterile tubes as desired. Inoculate all the 53 bacterial isolates separately and keep extra tube as an uninoculated comparative control.

Urease activity can be described as the splitting of urea via hydrolysis by a urease enzyme. The end products from this reaction yield ammonium carbonate and ammonia, which are alkaline in nature. The consequent rise in the pH of the medium is detected by phenol red indicator. The test is non-toxic, and the pH change that occurs from accumulation of alkaline end products is detected by a pH indicator in the media

Catalase activity

Collect all the bacterial isolates (53) from actively growing culture and pick up one colony with a sterile inoculating loop (of plastic or platinum) and apply the bacteria on a microscope slide.Add on drop of 3% H_2O_2 to the bacteria and observe the suspension. The bubbles resulting from production of oxygen gas clearly indicate a catalase positive result. The sample on the right below is catalase positive. The *Staphylococcus* spp. and the *Micrococcus* spp. are catalase positive. The *Streptococcus* and *Enterococcus spp. are* catalase negative.

Hydrogen Sulphide Production test

H_2S is commonly called as "rotten egg" gas, because of the copious amounts liberated through reduction of sulphur containing amino acids or through the reduction of inorganic sulphur compounds like sulphates, sulphites or thiosulphates or H_2S production can be detected by incorporating a heavy metal salt containing iron or lead ion as H_2S indicator to the medium. H_2S is a colorless gas when produced reacts with metal salt forms visible black insoluble ferrous sulphide precipitates causing blackening of the medium.

Weigh 6.4 gms of TSI media (make HI-MEDIA) and dissolve it into 100 ml of distilled water; and autoclave at 121$^oC$for 15 min.After autoclaving cool to 50$^oC$and pour into 4 sterile tubes to make slants, plug with cotton and allow solidifying.Inoculate three tubes with culture, keeping one tube as un inoculated comparative control.Incubate the tubes at 35-36^oC for 48 hours

Carbohydrate fermentation test

Phenol-Red Carbohydrate (PR-Carb) Fermentation Broth

It is useful for helping to characterize bacteria based on their fermentation abilities. Each PR-Carb broth contains the following: Carbohydrate (Each broth contains a single fermentable carbohydrate (glucose, lactose, sucrose and mannitol were used in this study).There are other organic nutrients (such as amino acids) that are not fermentable but can be used for growth. Phenol-Red this is a pH indicator that is RED at pH 7 or higher (alkaline) but turns YELLOW at low pH (acidic). The broth is initially pH neutral. So, if fermentation occurs, and acid by-products are formed, the solution will turn yellow. Durhamn Tube This is a small test tube that is inverted (upside-down) in the broth. If gases are produced, they will be trapped in the Durham Tube and a gas bubble will form. All the bacterial isolates were inoculated separately into each carbohydrate tube prepared as mentioned above and incubated for 37 for 48 hours and observed for formation of acid/gas.

Growth of different physiological conditions (Temperature, pH, salt tolerance)

Salt (2,5,and 7%), pH (5, 8 and 10.0.) and temperature (30° C, 40° C and 45° C) tolerance in nutrient broth adjusted to pH The media at different pH were inoculated with overnight grown inoculums (107 cells/mL), incubated at 37°C, 120 rpm for 24 h and cell growth determined by measuring absorbance at 660 nm. Temperature tolerance salt-tolerant cultures were used to examine their temperature tolerance by inoculating equal volume of overnight grown culture (107 cells/mL) in nutrient broth and incubating at temperatures ranging from 30°C, 40 ° C and 45° C for 24 h. and cell growth determined by measuring absorbance at 660 nm.

Antibiotics resistance and susceptibility of coliforms

Disc diffusion method

The disk-diffusion method (Kirby-Bauer) is more suitable for routine testing in a clinical laboratory where a large number of isolates are tested for susceptibility to numerous antibiotics. An agar plate is uniformly inoculated with the test organism and a paper disk impregnated with a fixed concentration of an antibiotic is placed on the agar surface. Growth of the organism and diffusion of the antibiotic commence simultaneously resulting in a circular zone of inhibition in which the amount of antibiotic exceeds inhibitory concentrations. The diameter of the inhibition zone is a function of the amount of drug in the disk and susceptibility of the microorganism.

This test must be rigorously standardized since zone size is also dependent on inoculum size, medium composition, and temperature of incubation, excess moisture and thickness of the agar. If these conditions are uniform, reproducible tests can be obtained and zone diameter is only a function of the susceptibility of the test organism. Zone diameter can be correlated with susceptibility as measured by the dilution method. Further correlations using zone diameter allow the designation of an organism as "susceptible", "intermediate", or "resistant" to concentrations of an antibiotic which can be attained in the blood or other body fluids of patients requiring chemotherapy.

Procedure:
Make a suspension at an appropriate turbidity of the bacterial culture to be tested.Place a sterile cotton swab in the bacterial suspension and remove the excess fluid by pressing and rotating the cotton against the inside of the tube above the fluid level. The swab is streaked in at least three directions over the surface of the Mueller-Hinton agar to obtain uniform growth. A final sweep is made around the rim of the agar. Be sure to streak for confluence.Allow the plates to dry for five minutes.Using sterile forceps, place disks containing the following antibiotics on the plate: penicillin-G, ampicillin, cephalothin, erythromycin, tetracycline, methicillin, streptomycin or other appropriate antibiotic disks.Incubate the plates within 15 minutes after applying the disks. The plates should be incubated soon after placing the disks since the test is standardized under conditions where diffusion of the antibiotic and bacterial growth commences at approximately the same time.Based on different biochemical tests, the ten isolated coliforms were identified upto genus level and one isolate WMF1 was sent for molecular identification. using 16s r RNA sequence.

Molecular identification of WMF1 using 16s r RNA sequence
DNA was isolated from the culture provided by the kit purchased from Xcelris, Ahmedabad. Quality was evaluated on 1.2% Agarose Gel, a single band of high-molecular weight DNA has been observed.Fragment of 16S rDNA gene was amplified by PCR using 8F and 1492R from the above isolated DNA. A single discrete PCR amplicon band of 1500 bp was observed.The PCR amplicon was purified and further process for the sequencing. Forward and Reverse DNA sequencing reaction of PCR amplicon was carried out with 704F and 907R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl GeneticAnalyzer.Consensus sequence of 1474 bp 16S rDNA gene was generated from forward and reverse sequence data using aligner software.The 16S rDNA gene sequence was used to carry out BLAST alignment search tool of NCBI genbank database. Based on maximum identity score first fifteen sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 5.

Molecular characterization of genes specific to *E.coli*
Genomic DNA was isolated using kit from xcelris for detection of *Lac Z* as the β-galactosidase gene and *uidA* beta-D-glucuronidase of *Escherichia coli.*

PROCEDURE
1.5ml of full-grown, overnight culture is centrifuged for 2minutes at 13000rpm.Supernatant was discarded.The pellet is resuspended in 500µl of TE buffer by repeated pippeting.Add 4µl of lysozyme and 3µl of protenase-K and mixed well. Then 10%SDS 30µl was added and mixed well by repeated pippeting.The mixture was incubated at 37°c for 1hour.Added 100µl of 5m Nacl and mixed well and Incubated for10minutes at 65°c.Added equal volume of phenol: chloroform:Isoamylalcohol.Centrifuge the mixture at 14000rpm at 4°c.Transferred the supernatant to a new tube.Add 0.6 volumes of Isopropanal and shaker tubes back and forth.Spin the eppendrof tube with mixture at 4000rpm at room temperature for 10-15minutes.Diacard the supernatant wash pellet by adding same volume of 70% ethanol.Respin at same condition.Discard supernatant.Air dried pellet.Redissolve pellet in 30µl of TE buffer 4°C.17. The purify of isolated DNA was checked by agarose gel electrophoresis.

LacZPrimer_F _ TGGCTATCTACAGCTACCAGCA

LacZPrimer_R _ GGGTTTGCCGACAATTAAAATA

Uid A_ Primer_F_ CTCCTACCGTACCTCGCATTAC

Uid A _ Primer_R_ ACGCGCTATCAGCTCTTTAATC

PCR for detection of Lac Z and uid A genes
Add 23.5 ul of sterile double distilled water into a 0.5 ml PCR tube.Add 28.5 ul of 10x Taq polymerase assay buffer.Add 5 ul of 25 mM Magnesium Chloride.Add 1.5 ul of 10 mM dNTP mix solution.Add 1 ul of template DNA (1 ng/ul).Add 2 ul each of forward and reverse primers.Add 5 ulTaq DNA polymerase (2.5U/ul).Mix the solution gently.Layer the reaction mix with 15 ul of mineral oil to avoid any evaporation.Carry out the amplification using following reaction conditions for 40 cycles. Initial Denaturation 94^0 C for 1 minute.Denaturation 94^0 C for 45 Sec.After the reaction is over take out the reaction mix and run 20-30 ul of the aqueous layer in 1.2% agarose gel.

Preparation of 1.2% Agarose gel and Electrophoresis:
Prepare 1x TAE by diluting required amount of 50Xtae buffer (1 in 50) with deionized or distilled water.Take 30 ml of 1xTAE in a 250 ml conical flask. Add 0.36g of agarose. Boil the mixture; dissolve agarose until a clear solution of agarose is formed. Swirl the mixture to mix and let it cool to 60^0 C for safe handling. Mean time seals the ends of the gel tray with tape.

Place the comb at one end of the tray, making sure it dose not touch the bottom of the tray but is close to it.If you want to view DNA under UV Transilluminator add 5 ul of ethidium bromide (stock 10 mg/ml). Swirl to mix.Pour enough agarose, slowly into the gel tray to cover the lower third of the comb. Care should be taken not to create any air bubbles. Allow the agarose to cool (it will become whitish and opaque).

RESULTS AND DISCUSSION

Biochemical tests: **Indole production test**

Observation: Development of cherry (deep) red color in the top layer of the tube is not observed.

Hence, *isolated organism* an indole – negative bacterium

Result: As development of cherry red color is not observed in the top layer of the tube so *Isolated organism* is negative test

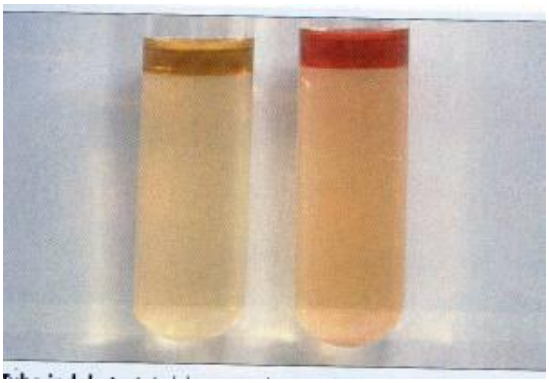
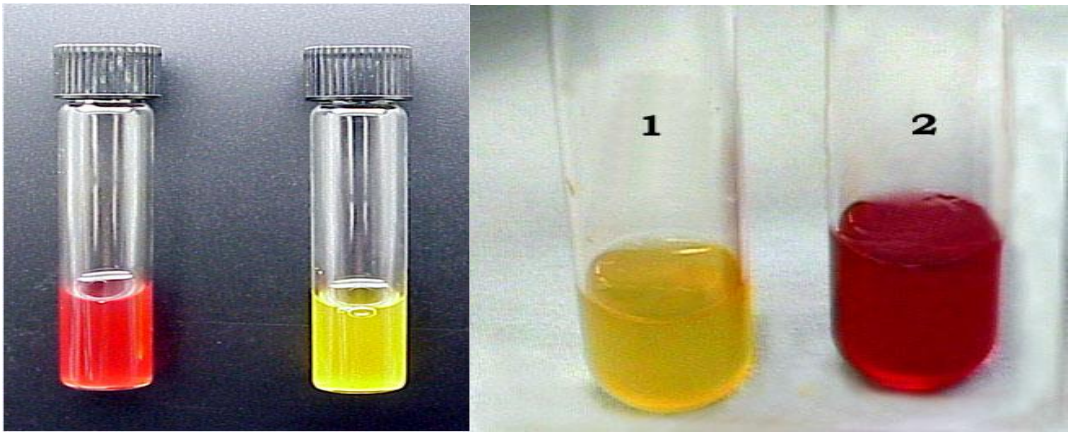


Fig 1: Indole test

Methyl-red and voges-proskauer tests

Observation: The tubes in which methyl red was added no red color was observed in the V-P test tubes when V-P reagents I & II were added no red color was observed.

Result: As in the methyl red test red color is observed hence, it is positive test. In the VP test, red color is not observed hence, it is negative test.



MR **VP**

Yellow-*negative* Red –*positive*

Fig 2: MR and VP test

Citrate utilization test

Observation: After 48 hours of incubation it was observed that there is no change in the medium color.

Result: From the above observation it is said that isolated organism is negative to this test.

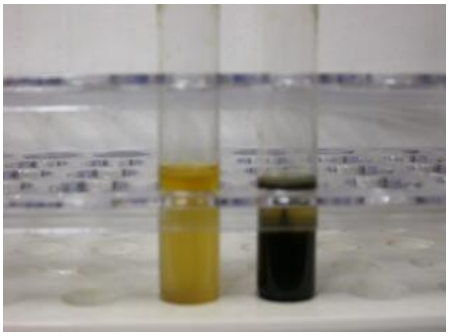
Green-*negative* Blue –*positive*

Fig 3: Citrate test

Hydrogen sulphide production test

Observation: No black coloration along the line of stab inoculation was observed (Figure 4).
Result: Black coloration along the line of stab inoculation was not observed. Hence the

Organism may be H_2S negative



Yellow-*negative* Black – *positive*

Fig 4: H_2S test

Catalase activity

Observation: After 48 hours of incubation when four drops of hydrogen peroxide was added to the slants slow appearance of gas bubbles was observed
R**esult:** After the addition of hydrogen peroxide gas bubbles were observed which the indication of positive test is. Hence *isolated organism i*s positive for catalase test

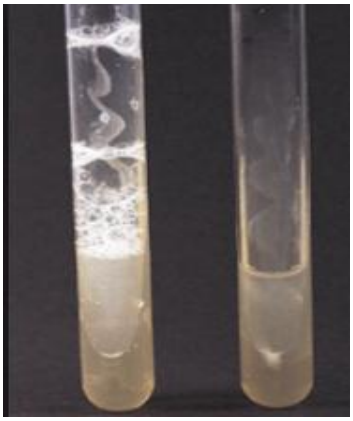


Fig 5: Catalase test

Urease test

Observation: After 48 hours of incubation it was observed that there is no change in the medium.
Result: From the above observation it is said that *isolated organism* shows positive test

Yellow-negative Pink – positive

Fig 6: Urease test

Table-1: IMVIC test results of bacterial isolates

S. No	Sample	Indole test	MR test	VP test	Citrate test
1.	WMF1	+	+	-	-
2.	WBB2	+	+	-	-
3.	WTT3	+	+	-	-
4.	WST4	+	+	-	-
5	WGF5	+	+	-	-
6.	WTB6	-	-	+	+
7.	WSB7	-	-	+	+
8.	WMUF8	-	-	-	+
9.	WGUF9	-	-	-	+
10.	WKUF10	-	+	-	+

(+= positive and - = negative)

Table-2: Other biochemical Tests

S. No	Sample	H_2S	Catalase	Urease
1.	WMF1	+	+	+
2.	WBB2	+	+	+
3.	WTT3	+	+	+
4.	WST4	+	+	+
5	WGF5	+	+	-
6.	WTB6	-	+	+
7.	WSB7	+	+	-
8.	WMUF8	-	+	+
9.	WGUF9	-	+	+
10.	WKUF10	+	+	+

(+= positive and - = negative)

Table 3: Carbohydrates fermentation

S/No	Sample	Glucose	Lactose	Manitol	Sucrose
		A G	A G	A G	A G
1	WMF1	+ +	+ +	- -	+ -
2	WBB2	+ +	+ +	- -	+ +
3	WTT3	+ +	- +	- -	+ -
4	WST4	+ +	+ +	- -	+ -
5	WGF5	- -	- -	- -	- -
6	WTB6	+ +	+ -	- -	+ +
7	WSB7	+ +	+ -	- -	+ -
8	WMUF8	+ +	+ -	- -	+ +
9	WGUF9	+ +	+ -	- -	+ +
10	WKUF10	+ -	+ -	- -	+ -

(A= acid formation, G= gas formation += Positive and -= negative)

Table 4: Bacteria tolerance on PH, Temperature and Salinity

S/ No	Sample	Effect of temperature			Effect of pH			Effect of salinity		
		30°C	40°C	45°C	5	8	9	2%	5%	7%
1	WMF1	+	+	-	+	+	-	-	-	-
2	WBB2	+	+	-	+	+	-	-	-	-
3	WTT3	+	+	-	+	+	-	-	-	-
4	WST4	+	+	-	+	+	-	-	-	-
5	WGF5	+	+	-	+	+	-	-	-	-
6	WTB6	+	+	-	+	+	-	-	-	-
7	WSB7	+	+	-	+	+	-	-	-	-
8	WMUF8	+	+	-	+	+	-	-	-	-
9	WGUF9	+	+	-	+	+	-	-	-	-
10	WKUF10	+	+	-	+	+	-	-	-	-

Antibiogram of Bacterial isolates

Antibiotic sensitivity is the susceptibility of bacteria to antibiotics using gentamycin, streptomycin, oflaxacin, norfloxacin and amoxicillin. Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating coliform bacteriainfection *in vivo*. Testing for antibiotic sensitivity is often done by the Kirby-Bauer method. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, is seen around the disc indicating poor growth. Antibiotic resistance and susceptibility: All the ten isolates showed variation in resistance and sensitive to the five antibiotics used.

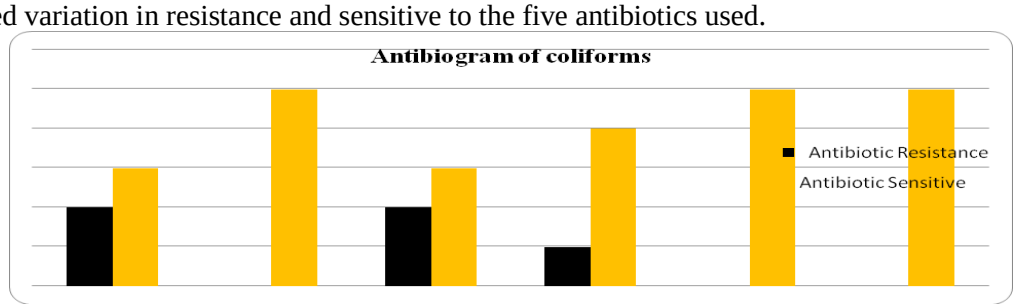


Figure 7: Antibiogram of coliforms

Table 5: Antibiogram of coliforms

S/ No	Sample	Bacteria	Gentamicin	Ofloxacin	Streptomycin	Norfloxacin	Amoxicillin
1	WMF1	E. coli	Sensitive	Sensitive	Sensitive	Resistance	Resistance
2	WBB2	E. coli	Sensitive	Sensitive	Sensitive	Resistance	Resistance
3	WTT3	E. coli	Sensitive	Sensitive	Sensitive	Resistance	Resistance
4	WST4	E. coli	Sensitive	Sensitive	Sensitive	Resistance	Resistance
5	WGF5	Proteus	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
6	WTB6	Enterobacter	Resistance	Sensitive	Sensitive	Sensitive	Resistance
7	WSB7	Klebsiella	Sensitive	Sensitive	Sensitive	Sensitive	Resistance
8	WMUF8	Pseudomonas	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
9	WGUF9	Pseudomonas	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
10	WKUF10	Salmonella	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive

Molecular (16s rDNA) identification of WWM1

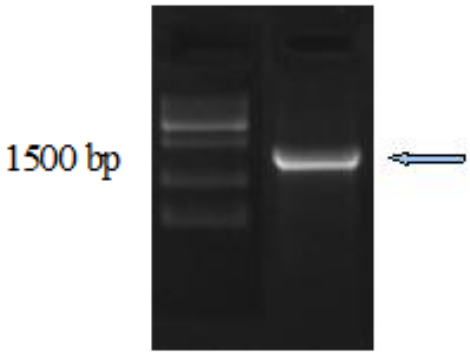


Figure: 8. 16s r DNA gel

0.8% Agarose gel showing single 1.5 kb of *16S rDNA* amplicon. Lane 1: 1 kb ladder; Lane 2: *16SrDNA* amplicon (WMF1)

Genome sequence revealed it as 99% similarity with *Escherichia coli* /*Escherichia fergusonii*

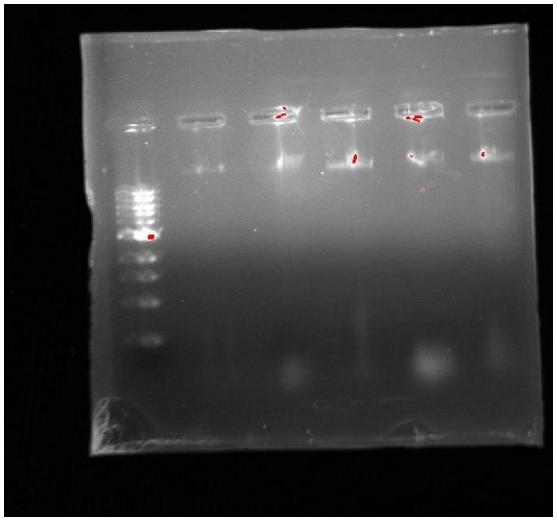


Figure 9: Genomic DNA isolation from WWM1

Lane 1: Marker Lane 2,3,4,5: Genomic DNA from WWM1

Detection of lac Z and uidA gene in *Escherichia coli* WWM1

Based on the primer 3 software, the designed primers for lac Z and uidA gene amplification revealed it to be around 500 bp lac Z and at amplified product of uid A gene is 147 bp. Our results also showed the presence of amplified product ~to 500 bp and lac Z as ~to 200bp.

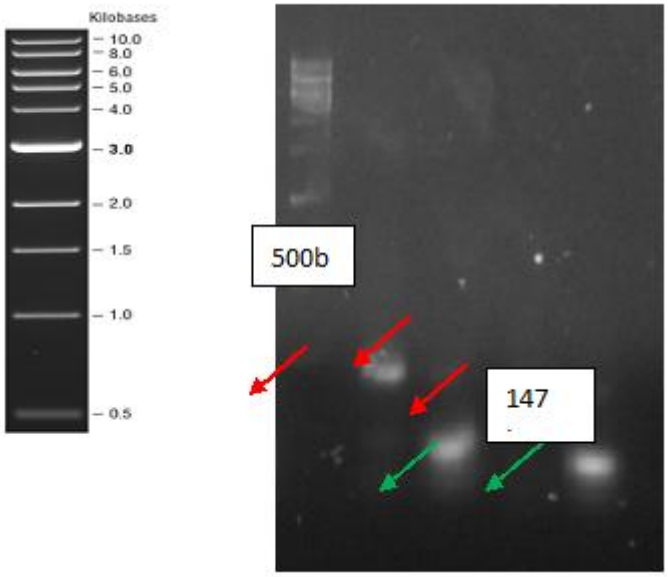


Figure 10: PCR amplification of Lac Z and uid gene

Lane 1: Marker Lane 2,4: Lac Z gene Lane 3,5: Uid A gene

The 16sRNA sequence was submitted to database and the accession no.KX361340 was allotted to the partial sequenceEscherichia coli strain WWM1 16S ribosomal RNA gene.

```
   1 gggcaacacgagcggggcgcatatggcttattttgttgaacgcgaggacgctacctggtc
  61 tcgacatccccccggaagttttcagagatgggaatgtgccttcgggaactgtgagacaggt
 121 gctgcatggctgtcgtcagctcgtgttgtgaaatgttgggttaagtcccgcaacgagcgc
 181 aacccttatcctttgttgccagcggtccggccgggaactcaaaggagactgccagtgata
 241 aactggaggaaggtggggatgacgtcaagtcatcatggcccttacgaccagggctacaca
 301 cgtgctacaatggcgcatacaaagagaagcgacctcgcgagagcaagcggacctcataaa
 361 gtgcgtcgtagtccggattggagtctgcaactcgactccatgaagtcggaatcgctagta
 421 atcgtggatcagaatgccacggtgaatacgttcccgggccttgtacacaccgcccgtcac
 481 accatgggagtgggttgcaaaagaagtaggtagctaaccttcggagggcgcttaccac
```

CONCLUSION

All these isolates were confirmed upto genus level using IMViC and other biochemical test such as urease, catalase, H_2S production and sugar fermentation. Anibiogram data of all the 10 isolates that grew on EMB agar and identified by biochemical tests were also cheked for antibiotic susceptibility and antibiotic resistance using antibiotics gentamycin, streptomycin, oflaxacin, norfloxacin and amoxicillin. One isolate WMF1 was identified using molecular (16sr RNA) as *E. colil Escherichia fergusonii*. Detection of two genes from WMF 1by PCR showed the presence of two genes. Lac Z corresponding to 500 bp and uidA gene corresponding to 147 bp confirming the presence of substrate utilizing genes in *E. coli* WMF1.

REFERENCES

Abdul, J., W. Abdul, R. Naseem, A. Hussain and Ali J. (2005). Comparative susceptibilities of *E. coli* to ceftriaxone alone and in combination with sulbactam. Pak. J. Med. Res. 44: 12-14.http://www.pakmedinet.com/7295

Abid, M.A. and Jamil, A. (2005). The Assessment of Drinking Water Quality and Availability in NWFP, NWFP, RWSSP, Peshawar.

Aihara, M. (1997). Infectious disease in Kenya: epidemiological study of diarrheal disease in children. Rinsho. Byori. 45 (5): 421- 6

Alhussain, J., A. Zahrani and A. Naeem, (2005). Susceptibility patterns of extended spectrum β-Lactamase (ESBL)- producing *E. coli* and *K. pneumoniae* isolated in teaching hospital. Pak. J. Med. Res., 44: 64-67. http://www.pakmedinet.com/8334

Alabaster, J.S and Llyod, R. (1980). Water quality for fresh fish. 1st edition. Butterworth London, p- 283.

Aldridge, K. E., Gardner ,B.B., Clark, S.J and Matsen, J.M. (1978). Comparison of Micro-ID, API 20E, and Conventional Media Systems in Identification of Enterobacteriaceae. J. Clin..Micrbiol. 7, 6: 507-513

Aljaro –García, C.; Bonjoch, X.; and Blanch, A. R. (2005). Combined immunomagnetic separation method and immunoblotting for the enumeration and isolation of *Escherichia coli* O157 in wastewaters.J. Appl. Microbiol. 98:589.

Alonso, M.C; Sanchez, J.M; Morinigo, M.A and Borrego, J.J. (1994). A direct membrane filters method for enumerating somatic coliphages in drinking water. Microbiol 10(3) 285-96

Andrei A. Zagorodni (2007). *Ion exchange materials: properties and applications*. Elsevier. ISBN 978-0-08-044552-6.

Bernhard A.E. and Field, K.G (2000a). Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes, Appl. Environ. Microbiol. 66:1587–1594.

Bej, A.K., Mahbubani, M.H. and Atlas, R.M.(1990). Detection of viable *Legionellapneumophila* in water by Polymerase Chain Reaction and gene probe methods. Appl.Environ. Microbiol. 57(2), 597-600.

Bej, A.K., Ng, W.Y., Morgan, S., Jones, D. and Mahbubani, M.H. (1996). Detection of viable *Vibrio cholerae*by reverse-transcription polymerase chain reaction (RT-PCR). Mole.Biotechnol. 5, 1-10.

Bernhard, A.E. and Field, K.G. (2000b). A PCR assay to discriminate human and ruminant feces on the basis of host differences in *BacteroidesPrevotella* genes encoding 16S rRNA, Appl. Environ. Microbiol. 66: 4571–4574.

Biswas, B.; Vemulapalli, R. and Dutta, S.K. (1994). Detection of *Ehrlichiaristicii* from feces of infected horses by immunomagnetic separation and PCR. J.Clin.Microbiol.32: 2147-2151

Blumenthal, U. J.; Peasey, A.; Ruiz-Palacios, G. and Mara, D. D. (1999). Guidelines for wastewater reuse in agriculture and aquaculture; recommended revisions based on new research evidence. WELL study, London School of Hygiene& Tropical Medicines and WEDC, London.

Bloch, K.C., Nadarajah,R., Jacob, R. (1997). *Chrysebacteriummeningosepticum*: an emerging pathogen among immunocompromised adults: Report of 6 cases and literature review. Med. 76:30-41

Cabello, F., Springer, A.D. (1997). {Typhoid fever in chili 1977-1990 an emergent disease}.Rev-Med-Chil .125(4):474-82.

Neemann, Jeff; Hulsey, Robert; Rexing, David; Wert, Eric (2004). "Controlling Bromate Formation During Ozonation with Chlorine and Ammonia". Journal American Water Works Association 96 (2): 26–29.

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