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ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF BACTERIA FROM COINS COLLECTED FROM DIFFERENT SOURCES

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ABSTRACT

Money is acting as a potential vehicle in the transfer of a various diseases, as it is being handled by people of various classes. Thus, this study was conducted to highlight the need for proper hygiene in order to avoid diseases that could arise due to uncleansed hands. In this study, coins were collected from 10 different sources: restaurant, street vendor, bakery, tea seller, fruit seller, butcher's shop, grocery, xerox shop, stationary shop and medicine shop. Collected coins were processed for the presence of cultivable bacteria. Identification of bacterial isolates was performed by culturing and biochemical investigations. Characterization was done by sequencing 16SrRNA gene. Results were found to bear major pathogenic organisms like *Escherichia coli*, *Pseudomonas stutzeri*, *Bacillus cereus*, *Salmonella enterica* etc. It witnessed visible presence of pathogens responsible for gastrointestinal infections in coins from places where people consume food while simultaneously handle money. The chances of cross contamination increases and in developing countries like India, poor hygiene practices after or while handling currency is the main reason behind maximum of the population suffering from stomach, nosocomial infections or any other forms of infection.

KEYWORDS: coins, contamination, money, pathogenic microorganisms, characterization.

INTRODUCTION

The potential role of currency in the spread of pathogenic microflora has been evaluated in many studies. Literature show that traces of mould, yeast, bacteria, faecal material, and even heroin and cocaine turn up on cash bringing new meaning to the term "filthy rich.". Cells of some of the bacterial strains will survive on copper surfaces for more than 48 hours. Remarkably, when these dry-surface-resistant strains were exposed to moist copper surfaces, resistance levels were close to those of control strains and MICs for copper ions were at or below control strain levels. This suggests that mechanisms conferring resistance against dry metallic copper surfaces in these newly isolated bacterial strains are different from well-characterized copper ion detoxification systems.[1]

Various studies largely focuses on the presence of bacteria on money, though some rarely cause any harm but we do come in contact with millions of pathogens daily in our life. Currency acts as a gold mine of disease-causing pathogens. As currency coins are handled by people of varying health and hygienic statuses, chances of pathogens being present on money should be acknowledged. Research shows that *E. coli* and *Salmonella enteritidis* can survive for upto eleven days and nine days respectively on the surfaces of coins and thus the potential of microbial transmissions via currency is quite high.[2] Other than being a possible source of gastrointestinal infections, immunocompromised individuals are at greater risk of acquiring nosocomial infections from a contaminated coin which was previously circulated in a hospital premises.[3] [4]

In this experiment, the main objective is to culture and isolate bacteria from coins. Further to identify these isolates which are potential pathogens and spread awareness for the necessity of proper washing of hands and the hazardous effect of simultaneous handling of money and food.

MATERIALS AND METHODS

Sampling

The coins were collected from 10 different local places: Restaurant, Street Vendor, Bakery, Tea Seller, Fruit Seller, Butcher, Grocery, Xerox shop, Stationary shop and Medicine Shop while preventing all possible chances of cross-contamination. The individuals from each of the shop were requested to drop a one-rupee coin into our sterile falcon tubes which were closed immediately. In the lab, liquid nutrient media was added into the falcon tubes to immerse the coin.[5]

Isolation of bacteria

The coins were kept immersed in the nutrient broth under continuous shaking conditions for 30 minutes and the coins were removed after 30 minutes of incubation, since they are made up of metals and can hinder the growth of bacteria. In order to achieve isolated colony, serial dilutions were performed. From each dilution made, 0.1 ml of inoculum was spread on to nutrient agar plates and kept at 37°C for 24 hours. Isolated colonies were observed after 24 hours and selected for sub culturing on fresh nutrient agar plates.

Identification of bacterial isolates

Bacterial colonies grown on solid media were visually inspected for colony growth characteristics. Gram staining was performed for all 14 different colony isolates and the slides were observed under 45x magnification for analysing the Gram nature and shape.

Further, the following biochemical tests were performed to identify the organism upto genus level.

a) IMViC

b) Catalase test

c) Motility

Bacterial identification using 16SrRNA

Genomic DNA isolation

Genomic DNA was isolated from 14 different colony isolates using DNA column based kit method**.** 6 ml of broth was taken and was centrifuged at 12,000 rpm for 10 minutes to obtain a thick pellet. 750µl of 1x homogenizing buffer was added into the vial and vortexed to dissolve the pellet, which was incubated at 65ºC for 20 minutes. 500 µl of lysis buffer was added and mixed well manually, followed by incubation for 20 minutes at 65ºC. The vials are next centrifuged for 10 minutes at 12,000 rpm to get rid of debris. After centrifugation, the supernatant was transferred into another tube and equal volumes of ice-cold isopropanol was added and mixed. In the next step, the solution was transferred through the silica based DNA columns. The columns were centrifuged at 12000 rpm for 1 minute. Which is followed by adding 750 µl wash buffer and centrifuged at 12,000 rpm for 1 minute for 2 rounds. These columns were kept for dry spin for 2 minutes to get rid of ethanol content. 50 µl of elution buffer was added and the tubes centrifuged at 12000 rpm for 1 minute. DNA thus obtained and further quantified.

Electrophoresis

The isolated DNA was quantified using the agarose gel electrophoresis. For electrophoresis, 1.2% agarose gel was prepared by adding 1.8 gm of agarose to 150 ml of 1x TAE buffer, and 10 µl of Ethidium Bromide. 2 µl of the isolated DNA samples were mixed with equal amount of gel loading dye and was loaded on gel keeping 1kb ladder as reference. After 35 minutes, the gel was taken out of the tank carefully and placed inside the gel documentation chamber and the genomic bands were viewed under ultraviolet light.

Amplification of 16srRNA gene and its purification

The reaction mixture for PCR amplification of the isolated DNA was set after Gel electrophoresis. The target gene was amplified using 27F-5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R-5'-GGTTACCTTGTTACGACTT-3' as forward primer and reverse primer respectively. Reaction mixture as shown in table was prepared and PCR plate was kept inside the thermocycler which was programmed to target the 16s rRNA gene; initial denaturation 95°C for 2 minutes, final denaturation 95°C for 30 seconds, annealing 50°C for 30 seconds, elongation 72°C for 1 minute for 30 cycles followed by final elongation 72°C for 10 minutes. After PCR amplification, entire reaction volume was run on gel to observe the amplified product. The PCR bands were cut precisely, dissolving in buffer and was eluted to obtain purified DNA for Sanger sequencing.

Sanger sequencing

After gel purification, the samples were sequenced bidirectional by performing sequencing PCR using the same primers which is followed by post sequencing PCR purification. The program used is as follows: initial denaturation 95°C for 2 minutes, final denaturation 95°C for 30 seconds, annealing 50°C for 30 seconds followed by termination 60°C for 4 minutes for 30 cycles. The purified plate was linked in 16 capillary genetic analyser (3130XL, Applied Biosystems). The obtained 16S rRNA sequences of 14 different cultures were queried against NCBI's sequence databases using on-line BLAST search.[6] Following, all the sequences were aligned using the Clustal omega computer program to understand the relationship of unknown sequence with other related species.[7] A phylogenetic tree was generated which informs us about evolutionary relationship and the phylogram to classify the organisms that are closely related to each other.

RESULTS AND DISCUSSION

Isolation and Identification of bacterial isolates

The coins collected from 10 different places produced 14 types of isolated colonies that differ from one another in their colony growth characteristics. They were further sub cultured on solid LB agar plates to obtained proper isolated colonies. Morphological analyses of colonies were done and are as mentioned in Table1. Based on the distinct colony characteristics 14 isolates were taken for further analysis. Microscopic examination of 14 isolates was performed using Gram's staining and listed in Table 2. Out of 14 isolates 5 were Gram positive rest showed characteristics of Gram negative bacteria. To further understand the properties of these bacteria few biochemical tests were conducted and results of which are as shown in Table 3. The results obtained were helpful in identifying the organisms upto genus level. The basic biochemical tests conducted were Indole test, Methyl red test, Voges Proskauer test, Citrate test (IMViC), Catalase test and Motility tests. To further identify organisms upto species level molecular identification was performed.

Table 1: Colony morphology of 14 isolates obtained.

Colonies	Colony characteristics							
	Form	**Elevation**	**Margin**	**Size**	**Surface**	**Opacity**	**Texture**	**Colour**
Bakery (Ba)	Circular	Raised	Entire	Punctiform	Smooth and glistening	Transl-ucent	Moist	Yellow
Butcher (Bu)	Circular	Flat	Entire	Medium	Smooth and glistening	Transl-ucent	Sticky	White
Stationary(Stn)	Circular	Raised	Entire	Punctiform	Smooth and glistening	Transl-ucent	Sticky	Pink
Grocery (G)	Circular	Flat	Entire	Punctiform	Smooth	Transl-ucent	Sticky	Yellow
Medicine Shop(MS)	Circular	Raised	Entire	Punctiform	Smooth and glistening	Transl-ucent	Moist	Yellow
Street Vendor (SV)	Filamen-tous	Flat	Filamen-tous	Large	Rough and glistening	Transl-ucent	Moist	Yellow
Xerox Colony No 1 (XeC1)	Circular	Raised	Entire	Punctiform	Smooth and glistening	Transl-ucent	Sticky	Yellow
Xerox Colony No 2 (XeC2)	Irregular	Flat	Undulate	Medium	Rough	Opaque	Powdery	White
Fruit Seller Colony No 1 (FSC1)	Circular	Raised	Entire	Punctiform	Smooth and glistening	Translu-cent	Sticky	Yellow
Fruit Seller Colony No 2 (FSC2)	Circular	Flat	Entire	Punctiform	Smooth and Dull	Translu-cent	Sticky	White
Tea Stall Colony No 1	Irregular	Umbonate	Undulate	Medium	Smooth and glistening	Opaque	Sticky	White

(TSC1)								
Tea Stall Colony No 2 (TSC2)	Irregular	Flat	Curled	Big	Smooth and Dull	Opaque	Moist	Brown
Restaurant Colony No. 1 (RC1)	Circular	Crateri -form	Entire	Medium	Smooth and Dull	Opaque	Sticky	White
Restaurant Colony No 2(RC2)	Circular	Crateri -form	Entire	Medium	Dull	Opaque	Dry	White

Table 2: Microscopic examination of 14 isolates obtained.

Colonies	Gram nature	Shape
Bakery (Ba)	Gram negative	Bacilli
Butcher (Bu)	Gram positive	Cocci
Stationary(Stn)	Gram negative	Bacilli
Grocery (G)	Gram positive	Cocci
Medicine Shop(MS)	Gram negative	Coccobacillus
Street Vendor (SV)	Gram negative	Cocci
Xerox Colony No 1 (XeC1)	Gram negative	Bacilli
Xerox Colony No 2 (XeC2)	Gram negative	Cocci
Fruit Seller Colony No 1 (FSC1)	Gram negative	Undefined
Fruit Seller Colony No 2 (FSC2)	Gram positive	Bacilli
Tea Stall Colony No 1 (TSC1)	Gram negative	Bacilli
Tea Stall Colony No 2 (TSC2)	Gram positive	Bacilli
Restaurant Colony No. 1(RC1)	Gram positive	Cocci
Restaurant Colony No 2(RC2)	Gram positive	Cocci

Table 3: States the biochemical tests results of IMViC, Catalase and Motility tests.

Colonies	Indole	Methyl red	Voges Proskauer	Citrate	Catalase	Motility
Bakery (Ba)	Negative	Negative	Negative	Negative	Positive	Non motile
Butcher (Bu)	Negative	Negative	Negative	Negative	Positive	Non motile
Stationary (Stn)	Negative	Negative	Negative	Negative	Positive	Motile
Grocery (G)	Negative	Negative	Negative	Negative	Positive	Non motile
Medicine Shop(MS)	Negative	Negative	Negative	Negative	Positive	Non motile
Street Vendor (SV)	Negative	Positive	Negative	Negative	Positive	Non motile
Xerox Colony No 1 (XeC1)	Negative	Negative	Negative	Negative	Positive	Non motile
Xerox Colony No 2 (XeC2)	Negative	Negative	Negative	Negative	Positive	Non motile
Fruit Seller Colony No 1 (FSC1)	Negative	Negative	Negative	Negative	Positive	Non motile
Fruit Seller Colony No 2 (FSC2)	Negative	Negative	Negative	Negative	Positive	Non motile
Tea Stall Colony No 1 (TSC1)	Negative	Negative	Negative	Negative	Positive	Non motile
Tea Stall Colony No 2 (TSC2)	Negative	Negative	Negative	Negative	Positive	Non motile
Restaurant Colony No. 1 (RC1)	Negative	Positive	Negative	Negative	Positive	Motile
Restaurant Colony No 2 (RC2)	Negative	Positive	Negative	Negative	Positive	Non motile

The genomic DNA isolated from cultures was run on gel for its quantification as shown in fig 1. The clear bands visualized under UV-light provide insight on how much volume of DNA should be taken further for amplification. 25-40ng of DNA was taken and programmed as explained above in materials and methods to obtain amplified bands as shown in fig 2. The amplified products thus obtained were subjected for gel purification to ensure the quality of gene sequence.

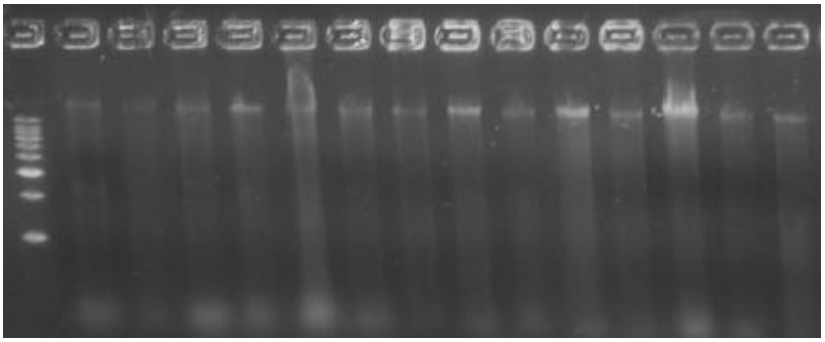

Fig 1: Genomic DNA of 14 different bacterial isolates in series: 1Kb ladder, Ba, Bu, Stn, G, MS, SV, XeC1, XeC2, FSC1, FSC2, TSC1, TSC2, RC1, RC2.

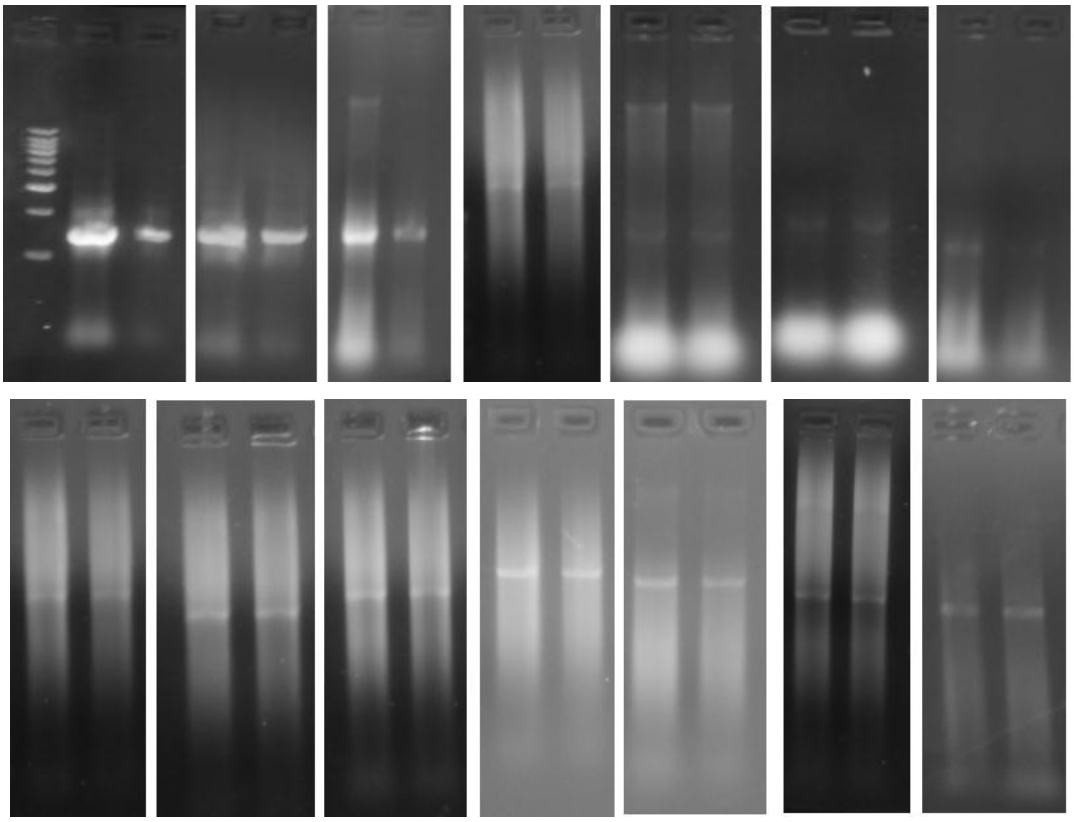

Fig 2: The PCR bands of amplified 16srRNA gene obtained in series: 1Kb ladder, Ba, Bu, Stn, G, MS, SV, XeC1, XeC2, FSC1, FSC2, TSC1, TSC2, RC1, RC2.

Sequencing files obtained were in .AB1 format which can be viewed by using software FinchTV. Quality of the obtained sequence can be observed through Electropherogram peaks as seen in fig 3. FASTA sequences for further analysis were obtained by converting .AB1 file in Seq Scanner 2.0. One Fasta sequence representing 14 samples is shown below. Using BLAST server unknown sequences were identified based on query coverage, percentage identity and e-value. Further phylogenetic analysis was done to find out the evolutionary relationship of our query sequence, representative trees are shown in fig 4 and fig 5.

>Query.ab1

GCAGTGCTCAGCTACACATGCAAGTCGACGGCAGCACAGGAAAGCTTGCTCTCT
GGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACTCTGTCGTGGG
GGATAACGTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGCA
GGGGATCTTCGGACCTTGCGCGATTGAATGAGCCGATGTCGGATTAGCTAGTTGG
CGGGGTAAAGGCCCACCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCA
GCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGG
CCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCTGGCTAATACCCG
GTTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCATCAGCC
GCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGC
GTAGGTGGTCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCA
GTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGCGGAATTCCTGGTGTAGC
AGTGAAATGCGTAGAGATCATGAGGAACATCCATGGCGAAGGCAGCTACCTGGA
CCAACACTGACACTGCTGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCC
TGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTTGGGTGCGATTTAGGCAC
GTCAGTATCGTAAGCCTGAACGCAGTTTAAGCTTTCGTTCAACCTGGGGCAGTAC
GGGTCAGCTAAGGACTGCAAACTGCATAAAGGACATCTGGACGGGAGAGGTGTC
TC.

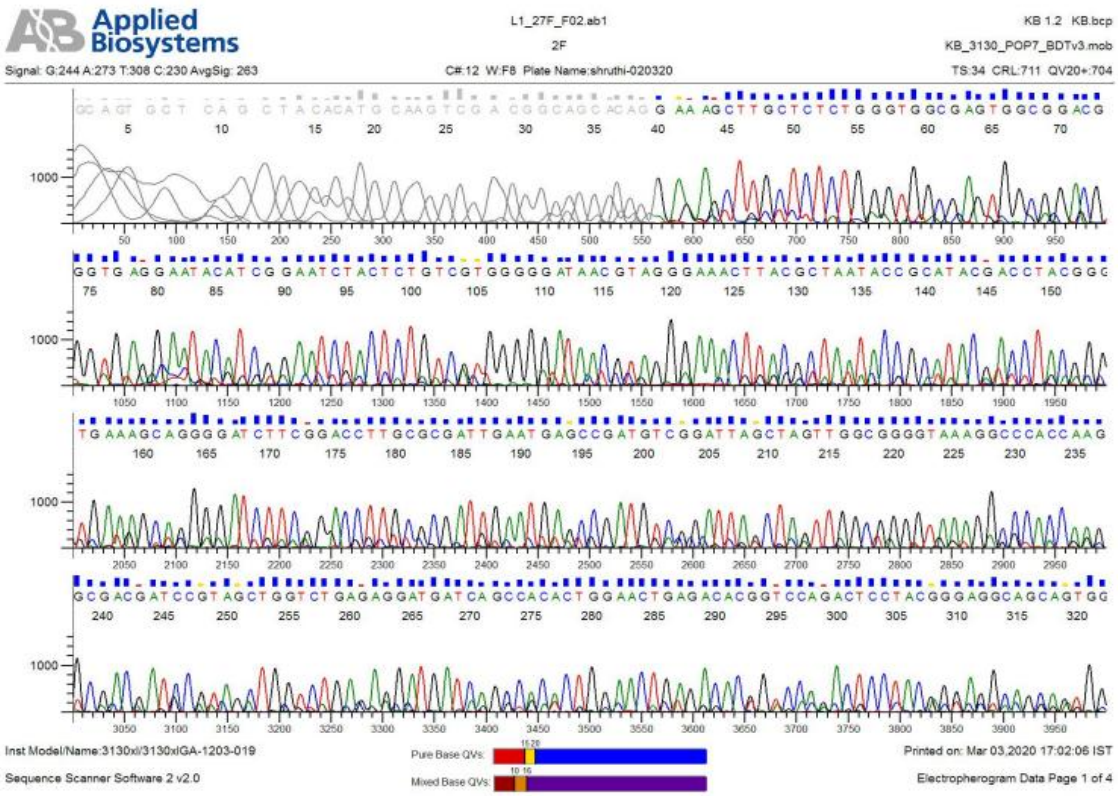

Fig 3: Sequence obtained in FinchTV showing the Electropherogram peaks- This is a representative file for 14 samples.

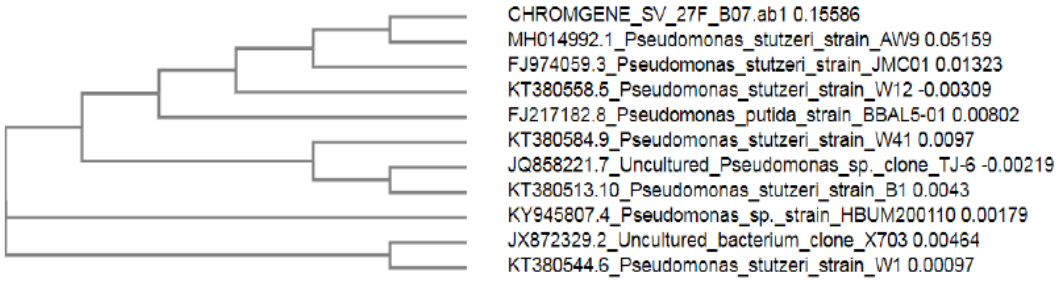

Fig 4: Phylogenetic tree obtained from Clustal Omega which depicts that Query sequence is closely related to *Pseudomonas stutzeri*.

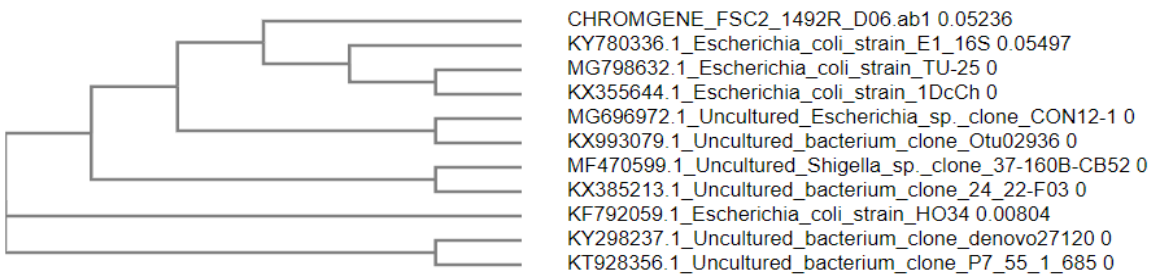

Fig 4: Phylogenetic tree obtained from Clustal Omega which depicts that Query sequence is closely related to *Escherichia coli*.

To summarize all the results obtained, using both the classical and molecular tools the colony isolates were characterized. Investigating, the Gram nature of the bacteria under study allows us to categorize the sample as Gram negative or positive. Along with Gram Nature of the bacteria, the morphology of the bacteria was also studied under microscope. Colony morphology also provides us with knowledge about the probable kind of bacteria by tallying the existing results with our own. Biochemical identification helps us to analyze the bacterial samples under study up to the genus by correlating with the properties of the bacteria obtained from the tests. The results were also confirmed by referring the Bergy's Manual and the results shown by the culture isolates, where inferred accordingly. The final step and probably the most precise way of identifying are the tools used in the molecular identification. The molecular identification stage was the most gradual process that needed skilled hands to perform as it did not allow any room for mistakes. Starting from isolating genomic DNA of the bacterial isolates to unravelling the DNA sequences, which was used to construct the Phylogenetic tree that helped us to identify our culture samples, every step was performed with utmost precaution.

Each and every coin sampled was found to carry nearly common bacteria. Causative agents of food poisoning like *Bacillus cereus* and *Salmonella enterica,* were isolated from coins collected from tea stall and butcher, respectively. A larger number of bacterial isolates like *Escherichia coli, Enterobacter* species, *Klebsiella pneumoniae* and *Salmonella enterica* clearly suggests faecal contamination from either an airborne source or hands to hands cross contamination. Studying the source of coins, viz, restaurants, fruit sellers and medicine shop, hand to hand cross contamination is the only logical answer for the presence of enteric bacteria.[5] Presence of *Pseudomonas* species clearly indicates the possibility of soil contamination or simply due to the fact it was handled by millions of people; but the presence of *Pseudomonas stutzeri* complicates it for it is majorly found in human spinal fluid. Certain rare bacteria like *Serratia* sp, *Chromobacterium violaceum, Actinobacillus pleuropneumoniae,* and *Streptococcus pneumoniae* were isolated from coins collected from tea stall, xerox shop, bakery and restaurant respectively. Isolation of *S. pneumoniae* from restaurant arises questions about its sanitation for it is generally bacteria that are responsible for nosocomial infections. *Serratia* sp., also a common pathogen behind nosocomial infection was isolated from the coin collected from stationary shop which may be due to personal hygiene of the shopkeeper. *Actinobacillus pleuropneumoniae,* isolated from the coin collected from bakery, is the leading cause of pneumonia in swine and humans generally

succumb to pneumonia after consuming infected meat. So in this scenario as well, the shopkeeper's personal hygiene is to be questioned.[8] [9] [10]

CONCLUSION

In conclusion, the study was successful in identifying coins as potential vector of diseases and the results highlights on the paramount of importance of hand sanitization when simultaneously handling of currency and food. The study also unfolds the perils of consuming foods from the streets or shops, where the practice of washing hands is considered as a luxury or unnecessary.

Apart from exposing the possible pathogens present on coins, the study revealed the extent to which pathogens can remain viable on the surface of the coin, thus ultimately aiming at the need of awareness for proper hygiene of the hands in order to ensure a healthy body. With the information the study shall provide, the scientific world might use the knowledge for devising drugs that inhibits the adapting mechanism of the pathogens under study. Further studies can be conducted to implement simple precautions everyone can follow to overcome the overall problem.

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