Summary

The microbial world is considered the largest unexplored reservoir of biodiversity on Earth. Understanding such diversity and its main causes could reveal phylogenetic relationships between organisms and address the question arose about the microbial interrelationships in their ecological niches and the emergence of new pathogenic traits. *Escherichia coli*; the best studied species in the microbial world represented a model for studying biodiversity in aquatic habitats. The environmental factors characteristic of water exert a great stress on biochemical, physiological, serological and molecular features of inhabitants. Selected parameters of water such as pH, temperature, turbidity, BOD, COD and DO were evaluated to aid in addressing attributations for *E.coli* diversity. In the study, three water sites were chosen (Well, river and drainage) based on expected variations in chemical and biological composition. The highest values were always exhibited by drainage water to indicate high pollution extend and meaningly, intense microbial community.Surprisingly,the highest population count for *E.coli* was found in river water samples to reveal sewage pollution of such site. *E.coli* was isolated from these sites using membrane filter technique and identified by two approaches; biolog system and 16s rRNA partial gene sequencing, but the last provided further sequence analysis and comparative studies. The isolates from well, river and drainage water were designated as *E.coli-EG1*, *E.coli-EG2* and *E.coli-EG3*, respectively.

Based on the expected dramatical changes that occur to any living flora in its habitat to accommodate the surrounding environment and evolve in such away that prevail and survive aiming to struggle for being kept alive, the studied isolates in their different niches undergo long term

evolution that is still inherited to next generations and short-term evolution that is temporary and lasts for short time periods. Traditional approaches such as cultural, biochemical, serological and phage typing were performed to reveal variability from different perspectives. Recent approaches such as sequencing and sequence analysis were adopted.

Image processing and analysis have come to enrich the information gathered about such diversity. The high population count recorded in river water matched with the extensive colonial growth found for *E.coli-EG2* on three types of media"macconkey, TSI and nutrient agar". In addition to such observation, the high oxidation capability and potential of such isolate gave it the chance to exceed the other two isolates. The data shown from CSU pattern ensured recalcitrant groups of carbon sources to be utilized. Concerning with antigenic properities that indicate the pathogenic ity and virulence capability on one hand, and on the other hand distinguish between different serotypes of *E.coli*.ouchterlony technique was performed using O-specific antisera and revealed close relations between *E.coli-EG1* and *E.coli-EG3* whilst *E.coli-EG2* exhibited a different pattern that attributed to novel antigenic features proposed by the law of nature.

Sensitivity to phage infection in water was governed by multiple factors that were reflected though data gathered from spot test and plaque assay which showed qualitative and quantitative discrimination among such isolates in such away that *E.coli-EG1* and *E.coli-EG2* showed positive response, but at differential extends.10^5 and 10^3 were recorded for *E.coli-EG1* and *E.coli-EG2* ,respectively. Upon digital image processing, the quantity of plaques shown numerically suggested very near similar sensitivity when taking plaques size and shape in consideration.

The uptake of the Congo red dye by *E.coli- EG2* and *E.coli- EG3* (although with differential pattern) indicated the expression of both virulence and Congo red binding genes and so, virulence. On the other hand, *E.coli- EG1* was considered a virulent.

Molecular approaches based on Multiple Sequence Alignment "MSA" of the first 500 nucleotides in the 16s rRNA gene had new insight, but from evolutionary and phylogenetic perspectives. The three *E.coli* isolates differed in the length, weight of partial sequences in which the shortest exhibited by *E.coli-EG2* that was attributed to mutational effect fundamentally, deletion and gene loss. The alignment revealed 57 variable sites and 42 singletons sites during comparison of nucleotides. The phylogenetic tree addressed the phylogenetic and evolutionary relations among such isolates in the way that *E.coli-EG1*and *E.coli-EG3* showed 97% and 93% to *E.coli-EG2*.

Nucleotide sequence alignment demonstrated different features such as length, weight, count of nucleotides, C+G, A+T, C+G/A+T and % C+G from which the last is the most important in classification and grouping of microorganisms in the same taxa.The highest melting temperature and % G+C were correlated and recorded for *E.coli-EG2*. Restriction pattern of DNA sequences showed similarity for *E.coli-EG1* and *E.coli-EG3* in no. of digested fragments, but at different lengths, whilst *E.coli-EG2* was dissimilar to the other isolates in fragment no. and length and recognition by ECO57MI activity which showed no such specificity for the other two sequences.

The fate of mutations (elucidated by MSA, restriction pattern and population genetic analysis) according to the neutral theory of molecular evolution (the Null hypothesis) is that those affect the fitness of their carrier is partly determined by natural selection. On one hand, new alleles that confer a higher fitness tend to increase in frequency over time until they reach fixation, thus replacing the ancestral allele in the population. This evolutionary process is called positive or directional selection. Conversely, new mutations that decrease the carrier's fitness tend to disappear from populations through a process known as negative or purifying selection. Finally, such alleles may tend to be maintained at an intermediate frequency in populations by way of the process known as balancing selection. The last case was described by The Tajima’s D test in which D value was positive to ensure that the evolutionary process for *E.coli* population accomplished by combination of both; mutation and genetic drift (the change in allele frequency for a specific gene within a population.

Image processing and numerical analysis represented novel approach for the evaluation of such diversity through a series of events; TEM imaging, image processing at grey level and RBG level and subsequent histographic analysis to convert images into digital ones. Numerical analysis regarding pixel intensities mean, median and standard deviation and no. of pixels for each bacterial cell of each isolate and their organelles was performed and showed highest composition of envelope and fimbrae for *E.coli-EG2*, cytoplasm and flagella for *E.coli-EG1* and nucleod for *E.coli-EG3*. Regarding the fact that nanotechnology exhibited two approaches; top-down approach and bottom –up approach, image processing is confined to the former category.

Image processing and analysis revealed for the first time stages of DNA replication from TEM images of *E.coli-EG1* in such a way that the fine stages, starting from single circular chromosome to two resulting separate chromosomes. Such pattern elucidated bidirectional DNA replication. The stages were shown at two to three dimensional structures.

As an example of bottom-up approach, the three *E.coli* isolates were examined for their capability to synthesize gold nanoparticles from gold ions. Such ability depends on the bioreduction and stabilization of colloidal nanoparticles resulted. The three isolates showed potential for the process; though at differential extend among the isolates.

All the data obtained from such study revealed diversification at different levels and from different perspective in correlation to the evolutionary and taxonomic impact and concerning to interpreting the behavior of all isolates in relation to their ecological niche. It appeared that almost the different approaches ensured the distinct behavior of *E.coli-EG2*, specially the molecular approach which suggests that it may be regarded as a novel species.

The overall diversity in *E.coli* was attributed to two phenomena; mutation and horizontal gene transfer. An assumption was derived for explaining such diversity by the aid of the natural selection theory and genomic potential model that regarded **"The evolution of *E. coli* as a model for prokaryotes may be explained in terms of adaptation by natural selection via accumulated mutations, genetic drift and genomic recombination in such away that only desired mutants get better chance for survivals while detrimental mutations is vice versa, taking in consideration that each species has its own origin and develops during the time course of life".**