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Escherichia coli, A Beneficial Bug, but a Dynamic Threat to Public Health: Call to Caution

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***Escherichia coli* is no doubt a bacteria with multitudinous application in research and industries, yet it has proven to be an intermittent threat to public health. Its application in biotechnology and biomedicine as veritable tool in the production of extracellular recombinant proteins and in synthesis of vital macromolecules and life sustaining proteins cannot be overemphasized. However, the impact of the various pathotypes in intermittent public health 'uproar' as recently experienced in eight European countries and as the agent of infant mortality in developing countries necessitates an update review of the bacterium. Antibiotic use in agriculture has been fingered as a selective force for the emergence of the antibiotic resistance noted among "superbug" isolates and call for concern. Large morbidity and mortality by the "superbug" *E. coli* reiterate the need for strict compliance to laboratory hygiene to prevent the spread of toxic *E. coli* genes into the environment. The presence of the superbug in ready-to-eat meals from the recent toxic *E. coli* outbreaks in France suggested post-cooking contamination and the need to brace up on personal and general hygiene.**

Key words: *E. coli*; Biotechnology; Pathotypes; Superbug; Public health; Hygiene.

Escherichia coli is a Gram negative non-spore forming facultative organism that is abundant in the gastrointestinal tract of humans and warm blooded animals[1]. The intestinal micro-biota consists of over 500 species of bacteria but *E. coli* is the predominant facultative anaerobe at this highly competitive site[2]. It has been suggested that its ability to utilize gluconate more effectively than other intestinal microorganisms has contributed to its immense success at existing in this unique environment[2-4]. This extremely versatile microorganism is probably the most studied organism in microbiology and it has become the model organism for many aspects of microbiological research[2,5] including but not limited to research in biochemistry, genetics and also the production of recombinant proteins[1]. *E. coli* can also be found in soil and water as a result of faecal contamination and this has led to its use as an indicator organism for poor water and/or food quality[6].

This ubiquitous bacteria colonizes the human infant gut within hours of birth to the first week of life after being acquired from the mother

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and other adult care givers[2,7] and it can live as a commensal in the human gut rarely causing disease, unless the host becomes immunocompromised or gastrointestinal barriers are breached[2]. *E. coli* is however far from being just a harmless intestinal organism or opportunistic pathogen; it can also be a highly virulent and fatal pathogen[6]. In the course of the evolution of this bacterium, it has acquired specific virulence factors which enable it to adapt to new environments or habitats and cause a variety of diseases. In this regard, different pathotypes of *E.coli* have been identified, over the past 50 years, based on the combination of virulence factors that they carry and these are responsible for the various types of infections attributable to the bacterium. Generally, the bacterium is responsible for intestinal or diarrhoeal diseases and extra-intestinal diseases of which urinary tract infections and meningitis in neonates are the most important[8,9]. These pathotypes and the newly emerging ones have been implicated in intermittent diarrhoeal epidemics that resulted in high morbidity and mortality around the globe[9-11].

In spite of the notable virulence of *E. coli* and its public health implications, its role as veritable tool in various spheres of life should not be by-passed. Hence, this article reviews the beneficial role of *E. coli* and emphasizes with its role as a ubiquitous pathogen with a call to alertness in view of the present realities.

E. coli as a beneficial bug: Usefulness in Biotechnology and Biomedicine

E. coli is of immense importance in modern biotechnology and industrial microbiology[12, 13]. This can be adduced to the ease of subjecting it to conventional laboratory analysis and genetic manipulation[14]; largely due to such qualities like safety in handling (when compared to the like of *Mycobacterium* spp.), simplicity, well known genetic properties, as well as its ability to pick up foreign DNA following already established techniques[15] that are characteristic of the bacteria. Thus, *E. coli* is undoubtedly the original bacterium used for recombinant DNA and biotechnology[16]. It serves as a veritable tool in the production of extracellular recombinant proteins, which command preference to intracellular production that does not require disruptions of cell during protein recovery[17].

The slow cycle time of yielding biodiesel from plants compared to the increasing demand for the commodity[18] necessitates the use of microbes[19], of which *E. coli* is an example. This is because microbes, in general, have short life cycles and higher turn-over rates[20]. Genetically manipulated *E. coli* has been employed in biodiesel production especially as it does not produce unwanted glycerol, unlike the engineered oil seed from plants. Lu *et al.,*[15] manipulated the genome of a fatty acid producing *E. coli* and observed the desired overproduction of 50% pure fatty acid easily convertible to biodiesel.

Many life-sustaining proteins and enzymes take their origin from *E. coli*[16]. An example is insulin which is a vital hormone-substitute for patients with diabetes mellitus[21]. To achieve insulin production, insulin gene is usually cloned into a suitable vector (*E. coli* bacterium cell), to produce the type that is chemically identical to the humans'[22]. This production of hormones is as good as the production of enzymes by the same organism. The enzymes produced by *E. coli* are utilized in the industrial production of other polymers, for example, by fermentation[13, 22]. Using its hydrolase enzymes through tricarbocylic acid cycle and Embden-Meyerhof pathway for example, *E. coli* has been applied in industries to convert glucose to hydroxy-L-proline[23]. Deng *et al.,*[24] were able to produce > 110 g l^{-1} of N-acetyl glucosamine by low-cost fermentation involving *E. coli*. Also, due to the degradative potentials and high productivity quotient, the bioengineered *E. coli* has been used extensively to remediate the polluted environments and synthesis of vital macromolecules respectively[16, 21].

One *E. coli* strain, *Escherichia coli* Nissle[17,19], belonging to the same classification scheme as others, is placed in O and H serogroups and confers non specific immunity as well as diverse therapy[25-27] on its host. This *Escherichia coli* Nissle exhibits microbial antagonism against Salmonella and *Candida albicans* thereby protecting the host against infection[28, 29]. It is therefore being applied as a probiotic in prophylactics and in therapeutic control of infection, diagnosis and treatment of malignancy[30] as well as bowel diseases[26]. This, owing to the fact that the probiotic strain has the ability to deliver therapeutic molecules which address basic human

body disorder *in vivo*[31]. This beneficial application was demonstrated earlier with *in vitro* study cell-in-line cultures where the *E. coli* prevents the invasion by *Yersinia enterocolitica, Listeria monocytogenes, Listeria pneumophila, Shigella flexneri*, Salmonella and virulent *E. coli*[32, 33]. An elevated therapeutic success rate of 86.7% cases of intestinal and extra-intestinal disorders was observed in some records, while symptom improvement was observed by 84.4% professional and 81.2% by non professional[34].

Escherichia coli as an Indicator Organism

The coliforms and in particular *E. coli* are used as indicator bacteria[35, 36]. The detection of *E. coli* in a water source may be indicative of faecal contamination and high potentials of having other pathogens in the wate[37, 38]. The diversities of associating pathogens in a river, along with *E. coli* are determined by an interplay of factors, which may include the proximity to domestic waste and industrial effluents, the composition of the wastes being disposed into the river body, intrinsic ecology of the river and climatic factors[39]. These factors play a vital role in the choice of the source of water for domestic use by humans and for agricultural purposes.

Non pathogenic and pathogenic _E. coli_ strains

Pathogenic *E. coli* strains have been implicated as aetiologies of various forms of diarrhoea, utilizing virulence factors and genes that are absent in non-pathogenic strains[40]. These pathogenic strains are classified into pathotypes based on their mode of infectivity, clinical signs observed during infection and their virulence genes[41]. The widely acceptable pathotypes (predominantly implicated in intestinal diseases) include Verocytotoxigenic/Enterohemorrhagic *E. coli* (VTEC/EHEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAggEC), Diffusely adherent *E. coli* (DAEC)[42]. The extra intestinal pathotypes are: meningitis associated *E. coli* (MNEC) and uropathogenic *E. coli* (UPEC)[2,43]. There are several other pathotypes that have been identified but the mechanisms of pathogenicity of these are not well known yet[44]. EPEC, EHEC and ETEC have also all been implicated in diseases in animals and these strains make use of the same virulence factors as in humans including specific colonisation factors not found in human strains[2]. The ETEC and EAggEC do not exhibit any form of overlap with other pathotypes, unlike EHEC and and O157 strains which are a subset of VTEC known to be human pathogens. ETEC produces enterotoxins, and is responsible for persistent travellers' diarrhoea[44]. These toxins determine the virulence of ETEC by its effects against enterocytes' functions[45-47]. VTEC produces verocytotoxin (VT) which are similar to Shiga toxin (Stx) produced by *Shigella dysenteriae* and this informed the alternate reference of the *E. coli* as Shiga toxin producing *E. coli* (STEC). The VT produced by ETEC as well as its subtypes (O175 and EHEC) interferes with cell protein and determine the strain's pathogenicity. The toxins (which are classified into VT1, VT2, and VT2 subtypes) and *eae* gene cause lesion of the intestinal microvillus and the adherence of the *E. coli* to erythrocyte membrane[48].

Epidemiology of the _E. coli_ Pathotypes and New Toxic Strains in Perspectives

The VTEC with its subtypes EHEC and O157 are of great importance to human health[42, 49]. They originate from reservoirs like fresh juice, fruit salad, cheese, lettuce, etc which are mostly uncooked and are served as delicacies mostly in the West[49]. The spread may also be due to swimming in contaminated pools or drinking water harbouring the bacteria. O157 can also be contacted through contact with infected individuals or animal faeces during recreation. O157:H7 has been implicated in the hemolytic-uremic syndrome (HUS) epidemic in United States, Europe and Japan (provide ref). While it is generally known that over a hundred and fifty serotypes cause human disease[50], in Argentina and Australia however, it is the non-O157 VTEC/STEC[51, 52] that are increasingly noted for infection[53]. As large as 73,000 per annum of clinical cases involving serotype O157:H7 were observed in USA[54]. The serotype is not only responsible for HUS but also for HC[55, 56], which affects the individuals below the age of maturity and it depicts the pathogenesis of Shiga toxin 2 (Stx2) by this serotype[57]. More of this toxin is released by the organism as a defence mechanism, usually subsequent to detection of unfavourable *in vivo* environment created by antibiosis or immune systems[58-60]. This idea is pertinent and has been applied by some researchers in pathogenesis

and pathogenicity studies of the organism[61]. One may guess that the reported high level of toxicity in the strain O157 during an outbreak may be due to massive ingestion of phage-susceptible intestinal *E. coli* through a common food source. This is because some toxic *E. coli* strains which are reported to be tolerant to low pH and low water activity in food seasonings[62] are sometimes phage-susceptible[63]; the attributes that may accord unpredictable pathogenicity and pathogenesis to them[64].

The World Health Organization estimates that the global diarrhoeal diseases are responsible for around 2 million deaths per year (1.7 – 2.5 million deaths) making these the third highest cause of death due to infectious diseases worldwide and the majority of these mortalities occur in children under age 5 [11]. Every child is estimated to have around 3.2 diarrhoeal attacks per year but in developing countries this figure is much higher and can sometimes be up to 12 attacks per year[10]. This contributes to the high infant and child mortality rates in these countries. There is also evidence that the long term consequences of such a heavy burden of disease in childhood may include compromised fitness and productivity in adulthood[10]. The outbreak of toxic *E. coli* that claimed lives recently in Germany and made more than 1 500 others ill in eight European countries promotes the dynamism of *E. coli* as a pathogen capable of a severe pandemic, whenever basic rules of hygiene by individuals, communities, industries and medical laboratories are neglected. The discovery of this pathogen in frozen foods, hamburgers and other 'fast food'[10] supports a possible breach in hygiene. To date, no strain of *E. coli* is impervious to heat sterilization. So, their presence in cooked item leaves more to be desired.

E. coli Gut Ecology

E. coli is no doubt a flora of the alimentary canal[65]. It has a tendency to cause infection when there is a shift in natural balance that exists between commensals and pathogens[66]. More serious clinical manifestations associated with the new toxic *E. coli* strain in Germany is a pointer to the potentials of any microbe to mutate, acquire more virulent genes and emerge in epidemic proportions when the opportunity arises. Attendant symptoms denoting this trend may include bloody diarrhoea, strokes (when the bacteria invades the kidney), comas and sometimes seizure. The struggle for survival by *E. coli* in unconducive gut environments occasioned by a shift in body balance or arbitrary use of antibiotics might also fuel the trend of pathogenesis[67, 68]. Meanwhile, fundamental knowledge supposes that the ingestion might be through widespread consumption of fresh produce[69]. However, further events are gradually forming as eye openers to other likely sources of infections like contamination of ready-to-eat food by the handlers and non-adherence to strict rules of hygiene that spread the organism from their natural ecological niche into the food[70]. The use of probiotic supplements may keep the normal ecological balance of the gut stable and is preferred to arbitrary use of antibiotics[71].

General Pathogenesis

The process by which the *E. coli* pathovars cause disease has been extensively studied and they generally use a "multi-step scheme" of pathogenesis similar to that used by other mucosal pathogens which include colonization of the mucosal site, evasion of host defences, multiplication and eventually host damage leading to disease[2,72]. Pathogenic strains of *E. coli* possess several virulence factors that determine what pathovar they belong to and the mode by which they cause diseases. Most of these strains have particular adherence factors which enable them to colonise other mucosal surfaces such as the urethra and respiratory tract which are outside their normal environmental niche[2]. The adhesins form long appendages called fimbriae or pili which allow the bacteria to adhere to host cells and activate signal transduction pathways with the help of secreted toxins and proteins leading to invasion of the host cells, avoidance of normal immune responses by the host cell and successful colonization[2, 44].

This pathogenesis depends on the pathotype in question. Enteropathogenic *E. coli* is the major causative organism of diarrhoea in infants, especially in developing countries[73] and since its identification significant strides have been made towards understanding the mechanisms by which this group of *E. coli* causes disease[74]. The characteristic histopathologic feature of EPEC infections is the formation of attaching and effacing lesions; a process which involves intimate

attachment of the bacterium to the epithelial layer of the intestine leading to cytoskeletal changes with a build-up of polymerized actin beneath the adherent bacteria. This leads to effacement of the intestinal brush border microvilli and destruction of the apical enterocytes membranes[43]. This ability to form attaching and effacing lesions is conferred on the EPEC by the presence of group of genes encoded on a 35 kb pathogenicity island (PAI) referred to as the locus of enterocyte effacement (LEE)[44].

The EPEC are thought to initially bind intimately to the epithelial cells with the aid of an adhesin such as intimin and this leads to the activation of a type III secretion system with translocation of various effector proteins into the cells. These effector proteins include Tir, EspF, EspG and Map[44]. The binding of the bacteria with the host cell through the interaction of intimin with Tir leads to phosphorylation of Tir by several host tyrosine kinases having the effect of increased permeability due to loosened tight junctions[75]. The inflammatory response causes polymorphonuclear leucocytes to migrate to the luminal surface and trigger the adenosine receptor which in turn upregulates the galanin-1 receptor leading to increased host cell response to galanin, an intestinal secretion mediator. EPEC induced diarrhoea therefore results from multiple mechanisms which include "active ion secretion, increased intestinal permeability, intestinal inflammation and loss of absorptive surface area resulting from microvillus effacement"[2, 44].

Enterohaemorrhagic *E. coli* also belongs to the group of attaching and effacing pathogenic *E. coli*. Its normal habitat is the intestinal tract of cattle but it also colonizes the distal ileum and large intestine in humans[2]. It has been implicated in a number of outbreaks of gastroenteritis particularly in the developed world as the causative organism of bloody diarrhoea which when complicated can lead to haemolytic uremic syndrome which can be fatal. The O157:H7 is probably the most important and studied serotype of EHEC but several non-O157 serotypes have also been implicated in outbreaks worldwide[5, 49].

Most of the O157:H7 isolates harbour a 92 kb plasmid which encodes several virulence factors including adhesins by which the organism causes disease[41]. However, the main virulence mechanism of EHEC is Shiga toxin (Stx) also known as Verocytotoxin (VT) which is phage encoded and is the defining characteristic which sets apart the STEC from other pathotypes of which EHEC O157:H7 is a subset of this group[44]. Stx consists of two subgroups: Stx1 and Stx2 which share only about 55% amino acid similarity[6].

After ingestion, shiga toxins are produced in the colon and absorbed across the gut epithelium[76]. They then bind to polymorphonuclear leucocytes in the circulation and are released at the target organs via the glycoprotein receptors; globotriaosylceramides (Gb3) Stxs located on the endothelial cells of the brain, kidney and intestines[44, 76]. Following entry of the toxin into the cells, they interact with subcellular components and that leads to inhibition of protein synthesis and apoptosis[77]. The resulting damage is as a result of direct toxicity and the induction of local cytokine and chemokine production leading to occulusion of the microvasculature of the target organs. In the kidneys, this damage can lead to the development of haemolytic uremic syndrome which involves haemolytic anaemia, thrombocytopaenia and eventually acute renal failure[2]. Most strains also have the LEE pathogenicity island similar to that of the EPEC and mostly only these serotypes that possess the PAI are associated with disease in humans but there have also been reports of LEE-negative STEC serotypes implicated in disease which supports the presence of other virulence factors apart from Stx[78].

Several other possible virulence factors have been described for the non-O157 strains that may be responsible for their ability to cause disease in humans. It has been reported that they carry a 93 kb plasmid which encodes a protein, ToxB similar to the clostridium toxin and the lifA protein of EPEC[2]. The plasmid also encodes the RTX toxin, a serine protease, a catalase and the StcE protein. This StcE is thought to cleave the C1 esterase inhibitor of the complement pathway leading to tissue damage, oedema and thrombotic changes seen in EHEC infections[2].

Enterotoxigenic *E. coli* is the main cause of traveller's diarrhoea in travellers to the developing world and infants in those countries[6,73]. The symptoms of profuse watery diarrhoea are due to the production of one or two types of enterotoxin

by the organism referred to as heat-stable enterotoxin (HT) or heat-labile enterotoxin (LT). The LT is very similar in structure and function to the cholera enterotoxin produced by *Vibrio cholera*[79]. It has about 80% similarity with the cholera enterotoxin and also has a single A subunit and five identical B subunits. STs however are monomeric toxins which consist of two classes, STa and STb. They are unrelated to each other in structure and function and only those of the STa class have been implicated in human disease[73]. ETEC colonize the small bowel mucosa with the aid of proteinaeceous colonisation factors such as colonization factor antigen (CFA), coli surface antigen (CS) or putative colonization factor (PCF) and then elaborate enterotoxins leading to increased intestinal secretion[2]. Elaboration of the LT-1 toxin leads to activation of adenylate cyclase leading to increased cyclic adenosine mononphosphate (cAMP) intracellularly. This increase causes a decrease in sodium absorption by villous cells and active chloride secretion by the crypt cells leading to osmotic diarrhoea. STa binds to the cells with the aid of guanylate cyclase and this in turn leads to increased cyclic guanylate monophosphate (cGMP) levels and stimulates increased chloride secretion while inhibiting the absorption of sodium chloride and intestinal fluid. STb however causes loss of the epithelial cells of the intestinal villi and partial villous atrophy[73].

Enteroaggregative *E .coli* is the second most common cause of traveller's diarrhoea and is becoming increasingly recognised as a cause of endemic and epidemic diarrhoea all over the world. It usually causes watery diarrhoea but may be associated with mucus and blood[2]. They are basically described as *E. coli* that do not elaborate heat-labile (LT) and heat-stable (ST) enterotoxins and adhere to Hep-2 cells in a stacked brick pattern referred to as aggregative adhesion but the definition may incorporate both pathogenic and non-pathogenic clones of enteroaggregative *E.coli*[79].

The pathogenesis of enteroaggregative *E. coli* involves colonization and adhesion to the intestinal mucosa predominantly the colon with the aid of aggregative adherence fimbriae (*aaf*) and other adherence factors[80]. This is followed by production of thick mucus by the bacteria and host cell resulting in the formation of a biofilm on the surface of the enterocytes and then enterotoxins and cytotoxins are secreted by the bacteria. This induces an inflammatory response leading to mild but significant mucosal damage especially in the affected areas of the colon and ultimately intestinal secretion resulting in diarrhoea[73, 80-83].

EAEC carry a 100 kb plasmid which encodes the genes responsible for the aggregative adherence fimbriae[44] which are related to the Dr family of adhesins. Four variants of AAF have been described and though the specific receptors are not known, AAF/II has been shown to bind fibronectin[82, 84]. In addition to AAF, a flagellin protein found on the surface of EAEC causes the release of IL-8 which stimulates neutrophil transmigration across the epithelium which can also lead to mucosal damage and secretion of fluids[84, 85]. The enterotoxins produced by EAEC include: *Shigella* enterotoxin 1 (ShET1), an oligomeric enterotoxin present also amongst strains of *Shigella flexineri* 2a and thought to be contributory to the secretory diarrhoea in EAEC infections[2]. They also produce the enteroaggregative *E. coli* ST (EAST1) which is a 38-amino-acid homologue of the ETEC STa toxin and may also contribute to diarrhoea. However the gene that codes for EAST1 is also found on many commensal *E. coli* so the role of this enterotoxin in EAEC diarrhoea is still under question[86]. Several strains also secrete a plasmid-encoded toxin (Pet) which is an autotransporter with enterotoxic activity and it causes cytoskeletal disruption and rounding of the epithelial cells by cleaving the cytoskeletal protein, spectrin[44].

Several virulence factors of the EAEC are regulated by a single transcriptional activator referred to as *AggR* and epidemiological studies have shown the association of this regulon with disease. Kaper *et al.,*[2] suggested that the term 'typical EAEC' be reserved for strains that carry the *AggR* gene or at least a subset of the *AggR*-regulated genes and that the term 'atypical EAEC' should be used for strains that do not carry this regulon.

The strain *E. coli* O104:H4, identified as the strain responsible for the most recent outbreak of *E.coli* infection in Europe in May 2011, has been reported to be PCR-positive for the *AggR* gene which is typical for enteroaggregative *E. coli* and in addition to other genes also possesses the *aggA*

gene which encodes for the AAF/1 adhesin. It has also been reported that the outbreak strain are moderate to good biofilm producers when cultured in Dulbecco's minimum essential medium (DMEM) supplemented with 0.45% glucose which is typical and defining for EAEC strains. They therefore concluded that the outbreak strain is indeed a typical EAEC which has acquired a bacteriophage encoding Stx/VT, explaining its ability to cause haemolytic uremic syndrome in patients affected[87].

Enteroinvasive *E. coli* are obligate intracellular organisms and have neither flagella nor adherence factors. They invade the colon and pass through the microfold cells by transcytosis into the submucosa layer. Once they invade the sub-mucosa they evade the host defence mechanisms with the aid of various effectors thereby causing diarrhoea[44]. EIEC are closely related to *Shigella* spp. genetically and pathogenically and the genes required for the mechanism of pathogenesis are found on a large virulence plasmid found in both EIEC and *Shigella* species[41].

Diffusely adherent *E. coli* (DAEC) attach to Hep-2 cell monolayers in a characteristic diffuse pattern by producing a fimbrial adhesin, F1845, which belongs to the Dr family of adhesins[2]. The infection of an intestinal cell line by DAEC has been reported to impair the activity and reduce abundance of sucrase-isomaltase and dipeptidylpeptidase IV along the brush border leading to enteric disease[88].

Diagnosis and detection of the pathogenic *E. coli* strains

In cases of diarrheal disease, specific tests are required to ascertain the aetiology, as *E. coli* is among the normal gastrointestinal flora. Conventional culture system is not enough to detect pathogenic strains of *E. coli*; Polymerase chain reaction (PCR) is used[88-90]. Though enrichment culture of modified tryptone soya broth supplemented with 20 mg/l of the antimicrobial novobiocin is used to isolate EPEC, ETEC, VTEC/EHEC, EIEC, EAggEC and DEAC from faeces, appropriate detection require advanced techniques[91]. Serotypes of several EPEC colonies with polyvalent antisera need to be determined for identification while the adhesion to the tissue culture cells which can be demonstrated by a fluorescence actins staining test or DNA-based for the detection of attachment can reveal the strain to be EPEC[57, 92]. For VTEC O157, a combination of cultural systems and immunology may be used for detection. This involves the use of an immuno-magnetic separation (IMS) procedure that utilizes magnetic beads on which specific VTEC O157 antibody has been coated[51, 52]. The beads are inoculated to test for sorbitol non fermenters. The production of ST/VT and the attendant genes are detected biologically, immunologically and using molecular based (PCR) methods (e.g. for *eae* and *vtx*)[57, 92]

Presumptive positive isolates from conventional culture for ETEC may be confirmed using gene probes that are specific for heat labile (LT) and heat stable (ST) genes[92]. This can be used for direct detection from food and water samples. If EIEC is suspected, a tissue culture is used to assess the invasiveness or nucleic-acid-based assays for invasion-associated genes. This is unlike EAEC and DAEC which are identified strictly based on assays for aggressive or diffuse adherence in tissue culture.

To track the source of infection during an *E. coli* epidemic, just like the recent case in Germany, it is imperative to collect samples of ready-to-eat foods and portable water[93]. This is because such an outbreak is usually common source type. Laboratory confirmation may be carried out by DNA fingerprinting for the bacteria from various common sources[94]. For instance, a report from Northern France stated that eight children were rushed to the hospital following infection from beef burgers noted to have been contaminated with the *E. coli* strain, just as the frozen beef patties in France have been confirmed to harbour the bacteria[10]. Since most of these pathogenic strains of *E. coli* are readily available contaminants and are harboured in fruits and raw vegetable as their reservoir, regions where fast food and uncooked (salad) food are rampantly consumed are prone to their epidemics with little carelessness of the handlers.

Treatment of Diseases Caused by Pathogenic *E. coli*

The treatment of *E. coli* infections generally depends on the site and severity of infection. This decision is based on whether the infection is enteric or non-enteric and the mode of presentation of the illness. Infections caused by

intestinal *E. coli* present mainly as different types of diarrhoea depending on the pathotype responsible while the non-intestinal infections commonly include: meningitis, urinary tract infections, pneumonia, intra-abdominal infections and bacteremia. Oral rehydration therapy is the backbone of treatment of *E. coli* diarrhoea and it can be lifesaving if used effectively but it has no effect on the course of the disease and no antibacterial properties[95, 96].

The main goal of treatment of infections caused by the STEC group would be to limit the duration and severity of gastrointestinal symptoms, to prevent the onset of fatal systemic complications such as hemolytic uremic syndrome (HUS) and to prevent further spread of infection in the community[97]. Antibiotic treatment of enteric infections by STEC especially the O157:H7 serotypes remains a controversial issue as there are no appropriate advanced power randomised control trials to provide evidence based information on the benefits or otherwise of antibiotic use.

Several studies that have been done have concluded that there is no significant advantage of the use of antibiotic therapy and in fact, they may predispose to or increase the risk of development of HUS[61, 97]. However, Takeda *et al.,*[98] reported that timely commencement of antibiotics may reduce the duration of the illness and prevent progression to HUS. Nevertheless, there are other arguments that preclude the use of antibiotics. Use of antibiotics which cause bacterial cell lysis may lead to the release of even more shiga toxin into the lumen of the gut from where it can be absorbed into the systemic circulation or may cause induction of the bacteriophages carrying the *stx* genes which leads to increased production of the toxin[59]. Mora *et al.,*[99] also reported a high level of antibiotic resistance amongst STEC in Spain. Therefore, institution of an inappropriate empirical therapy could lead to overgrowth of the bacterium in the gut due to a selective advantage.

Anti-motility agents are also not encouraged in the treatment because they would prevent the elimination of the bacteria from the gut leading to prolonged exposure to the toxin and worsening the disease[100] or possibly leading to other neurological complications[101]. The main stay of treatment is therefore supportive with the goal being proper and adequate rehydration with replacement of electrolytes as indicated. Daily laboratory tests could also be done as suggested by Tarr *et al.,*[102] to monitor blood count, electrolytes, serum urea nitrogen and creatinine levels. The risk of developing HUS is considered to be past when the platelet count rises or if the platelet count is stable and symptoms are resolving[102]. On the other hand, treatment of infections caused by enteropathogenic and enterotoxigenic *E. coli* with appropriate antibiotics, shortens the clinical course of the disease considerably by reducing the duration of diarrhoea and excretion of the organism[103].

The role of antibiotics in the emergence of toxic pathogens

The toxic *E. coli* was reported to be resistant to multiple antibiotics[104]. Though the emergence of such resistance is not new[105], yet it might suggest that clinical strains have access to the environment, by and large, to food items. It might also be a result of the use of antibiotics in meat production that resulted in the emergence of such resistance[104]. Inappropriate use of antibiotics may create a non-conducive environment for bacteria, rather than eliminating them. The bacteria may therefore develop new features for adaptation and survival. Such features give them either a higher level of virulence or basically antibiotic resistance, hence the need to re-address the overuse of antibiotics in meat production[106].

CONCLUSION

The ubiquitous and versatile *E. coli* is no doubt an indispensable tool in research, with huge proceeds that have benefitted both human and animals. However, it represents a serious public health threat worldwide in terms of the potential to cause life threatening human diseases with high mortality rates. Numerous sporadic cases and major epidemics have been associated with *E. coli* in ground beef, apple juice, milk, lettuce, salami, and water, due to poor handling. Hence, prevention and control of *E. coli* causing human illnesses should be seen as a high-priority concern. Food handlers should be made to undergo compulsory training on hygiene. Extra care should be taken by researchers from all wards of life to prevent being the vehicle for transmission of toxic *E. coli* from the laboratory to the environment, which may be

difficult to control. An urgent international convention to address the excessive use of antibiotics in meat production, which has been spotted as a selective force for the emergence of toxic *E. coli* strains is hereby proposed. It hoped that an increase in public awareness, along with education associated with safe food handling practices and concerted efforts by all stakeholders are required to prevent and control toxic *E. coli* outbreaks.

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Competing Interests

None declared

REFERENCES

1. Donnenberg MS, Whittam TS. Pathogenesis and evolution of virulence in enteroopathogenic and enterohaemorrhagic *E. coli*. *J. Clin. Invest*. 2001; **107**(5): 539-548.
2. Kaper JB, Nataro JP, Mobley HLT. Pathogenic *Escherichia coli*. *Nat Rev Microbiol* 2004; **2**: 123-140.
3. Sweeney NJ. The *Escherichia coli* K-12 gntP gene allows E. coli F-18 to occupy a distinct nutritional niche in the streptomycin-treated mouse large intestine. *Infect. Immun*. 1996; **64**: 3497-3503.
4. Tenaillon O, Skurnik D, Picard B, Denamur E. The population genetics of commensal *E. coli*. *Nat Rev Microbiol.* 2010; **8**: 207-217.
5. Bettelheim KA. The non-O157 shiga-toxigenic (verocytotoxigenic) *Escherichia coli*; underated pathogens. *Crit. Rev. Microbiol.* 2007; **33**: 67-87.
6. Sousa CP. The versatile strategies of *Escherichia coli* pathotypes: a mini-review. *J. Venom. Anim. Toxins. Incl. Trop. Dis.* 2006; **12**(3): 363-373.
7. Bettelheim KA, Lennox-King S. The acquisition of *Escherichia coli* by new-born babies. *Infection* 1976; **4**: 174-179.
8. Komolafe A O, Adegoke A A. Incidence of bacterial septicaemia in Ile-Ife Metropolis, Nigeria. *Malays. J. Microbiol.* 2008; **4**(2): 51- 61.
9. Adegoke AA, Okoh AI. Studies on Symptomatic Urinary Tract Infection and Asymptomatic Bacteriuria in a University Community. *J. Pure Appl Microbiol*. 2012; **6**(1): (in press)
10. Savary P. *E. coli* outbreak sends eight kids to hospital. Galgary Herald. Downloaded by 10a.m. from http://www.calgaryherald.com/health/coli+ outbreak+sends+eight+kids+hospital/4962102/story.html#ixzz1PX5RmHTG on 2011; **06**: 17.
11. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull W. Health Organ* 2003; **81**: 197-204.
12. Lee SY. "High cell-density culture of Escherichia coli". *Trends Biotechnol*. 1996; **14**(3): 98-105.
13. Hassan S, Liu W, Ma, JK C, Thomas CR, Keshavarz-Moore E. Characterisation of mechanical properties of transgenic tobacco roots expressing a recombinant monoclonal antibody against tooth decay. *Biotech Bioeng* 2008;**100**: 803-809. DOI 10.1002/bit.21819.
14. Sawitzke JA, Thomason LC, Costantino N, Bubunenko M, Datta S, Court DL. Recombineering: In Vivo Genetic Engineering in *E. coli, S. enterica*, and Beyond. *Methods in Enzymology.* 2007; **421**: 171-199.
15. Lu X, Vorab H, Khosla C. Overproduction of free fatty acids in *E. coli*: Implications for biodiesel production. *Metabolic Eng* 2008; **10**(6): 333-339.
16. Cornelis P. "Expressing genes in different *E. coli* compartments". *Curr. Opin. Biotechnol*. 2000; **11**(5): 450-4.
17. Farmani J, Safari M, Roohvand F, Razavi SH, Aghasadeghi MR, Noorbazargan H. Conjugated linoleic acid-producing enzymes: A bioinformatics study. *Eur. J. Lipid Sci. Technol*. 2010; **112**: 1088-1100.
18. Romano M, Liversage H. Biofuels, land rights and community-investor partnerships. http://www.globalbioenergy.org/uploads/media/1010_IFAD_-_Biofuels__land_rights_and_community-investor_partnerships.pdf. 2010
19. Antoni D, Zverlov VV, Schwarz WH. Biofuels from microbes. *Appl. Microbiol. Biotech*. 2007; **77**: 23-35.
20. Esquenazia E, Jonesa AC, Byruma T, Dorresteina PC, Gerwick WH. Temporal dynamics of natural product biosynthesis in marine cyanobacteria. http://www.pnas.org/ content/108/13/5226.abstract 2011
21. Joshi SR. Newer Insulins: Making Early Insulin Initiation Safer and Simpler. CME downloaded on 11th June, 2011 from http://www.apiindia.org /postgraduate_ medicine _ 2004/chapter_41.pdf
22. Panahi M, Alli Z, Cheng X, Belbaraka L,

Belgoudi J, Sardana R, Phipps J, Altosaar I. Recombinant protein expression plasmids optimized for industrial *E. coli* fermentation and plant systems produce biologically active human insulin-like growth factor-1 in transgenic rice and tobacco plants. *Transgenic Res.* 2004; **13**(3): 245-59.

23. Ogawa J, Shimizu S. Industrial microbial enzymes: their discovery by screening and use in large-scale production of useful chemicals in Japan. *Current Opinion in Biotechnology*, 2002; **13**: 367-375.
24. Deng MD, Severson DK, Grund AD, Wassink SL, Burlingame RP, Berry A, Running JA, Kunesh CA, Song L, Jerrell TA, Rosson RA. Metabolic engineering of *E. coli* for industrial production of glucosamine and N-acetylglucosamine. *Metabolic Eng* 2005; **7**(3): 201-214.
25. Schultz M. Clinical Use of E. coli Nissle 1917 in Inflammatory Bowel Disease. *Inflamm Bowel Dis.* 2008; **14**(7): 1012-1018.
26. Schultz M., Butt G. *E. coli Nissle 1917 in the Treatment of Inflammatory Bowel Disease. Inflammatory Bowel Disease: A Practical Approach*, Series 2010; #60
27. Guttsches A, Loseke S, Sonnenborn U, Enders C, Gatermann S, Bufe A. Anti-inflammatory modulation of immune response by probiotic *E. coli* Nissle 1917 in human blood mononuclear cells. *Innate Immunity* 2011; doi: 10.1177/ 1753425910396251
28. Mandel L, Trebichavsky I, Splichal I. Stimulation of intestinal immune cells by *E. coli* in gnotobiotic piglets. In: Mestecky J, ed. *Advances in Mucosal Immunology*. New York: Plenum Press 1995; 463-464.
29. Lorenz A, Schulze J. Establishment of *E. coli* Nissle 1917 and its interaction with *Candida albicans* in gnotobiotic rats. *Microecol Ther*. 1996; **24**: 45–51.
30. Stritzker J, Hill PJ, Gentschev I, Szalay AA. Myristoylation negative msbB-mutants of probiotic *E. coli* Nissle 1917 retain tumor specific colonization properties but show less side effects in immunocompetent mice. *Bioengineered Bugs* 2010; **1**(2): 139-145
31. Loessner H, Leschner S, Endmann A, Westphala K, Wolf K, Kochruebe K, Miloud T, Altenbuchnerd J, Weiss S. Drug-inducible remote control of gene expression by probiotic *E. coli* Nissle 1917 in intestine, tumor and gall bladder of mice. *Microbes and Infection*. 2009; **11**(14-15), 1097-1105.
32. Boudeau J, Glasser AL, Julien S, Inhibitory effect of probiotic *E. coli* strain Nissle 1917 on adhesion to and invasion of intestinal epithelial cells by adherent-invasive *E. coli* strains isolated from patients with Crohn's disease. *Aliment Pharmacol Ther*. 2003; **18**: 45-56.
33. Altenhoefer A, Oswald S, Sonnenborn U, The probiotic *E. coli* strain Nissle 1917 interferes with invasion of human intestinal epithelial cels by different enteroinvasive bacterial pathogens. *FEMS Immunol Med Microbiol.* 2004; **40**: 223-229.
34. Rohrenbach J, Matthess A, Maier R, *Escherichia coli* Stamm Nissle 1917 (EcN) bei Kindern [*E. coli* Nissle 1917 in children]. Kinder Jugendarzt. 2007; **38**: 1-5.
35. CSIR Environmental Services: Pretoria. Department of Water Affairs and Forestry. 1996.
36. Rompre A, Servais P, Baudart J, de-Roubin MR, Laurent P. *J Microbiol Methods* 2002; **49**(1): 31-54 .
37. Hinton MH. Infections and Intoxications Associated with Animal Feed and Forage which may Present a Hazard to Human Health. *The Vet. J.*, 2000; **159**: 124-138.
38. Jenkins SR. Health effects of biosolids applied to land: available scientific evidence. http:// www.vdh.state.va.us/epidemiology/DEE/ documents/ Biosolidsfinal.pdf. 2007.
39. Odjadjare EEO. Prevalence of Listeria pathogens in effluents of some wastewater treatment facilities in the Eastern Cape Province of South Africa. A thesis in fulfilment of the requirements for the degree of Doctor of Philosophy in Microbiology, in the Faculty of Science and Agriculture at the University of Fort Hare. 2010
40. Anjum MF, Lucchini S, Thompson A, Hinton JCD, Woodward MJ. Comparative Genomic Indexing Reveals the Phylogenomics of *Escherichia coli* Pathogens. *Infect Immun*. 2003; **71**(8): 4674-4683.
41. Johnson JJ, Nolan LK. Pathogenomics of the virulence plasmids of *Escherichia coli. Microbiol Mol Biol Rev* 2009; **73**:750-774.
42. Erikson, E. Verotoxinogenic *Escherichia coli* O157:H7 in Swedish Cattle and Pigs. *Doctoral Thesis*. Swedish University of Agricultural Sciences. 2010.
43. Clarke SC. Diarrhoeagenic *E. coli* – an emerging problem? *Diag Microbiol Infect Dis* 2001; **41**: 93-98.
44. Croxen MA, Finlay BB. Molecular mechanisms of *E. coli* pathogenicity. *Nat Rev Microbiol.* 2010; **8**: 26-38.
45. Blanco J, Blanco M, Garabal JI, González EA. Enterotoxins, colonization factors and serotypes of enterotoxigenic *E. coli* from humans and animals. *Microbiologia* 1991; **7**(2): 57-73.

46. Nagy B, Fekete PZ. Enterotoxigenic *E. coli* (ETEC) in farm animals. *Vet Res.*1999; **30**(2-3): 259-84.
47. Nagy B, Fekete PZ. Enterotoxigenic *E. coli* in veterinary medicine. *Int. J. Med. Microbiol*. 2005; **295**: 443-454.
48. Alexandre M, Prado V. Detection of Shiga toxin-producing *E coli* in food. *Expert Rev. Mol. Diag*. 2003; **3**(1): 105-115.
49. Bettelheim KA. Non-O157 verocytotoxin-producing *Escherichia coli*: A problem, paradox, and paradigm. *Exp. Boil. Med*. 2003; **228**: 333-334
50. Griffin, PM. *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli*, p. 739–758. In M. J. Blaser, P. D. Smith, J. I. Ravdin, H. B. Greenberg, and R. L. Guerrant (ed.), Infections of the gastrointestinal tract. Raven Press, New York, N.Y.1995
51. Elliott EJ, Robbins-Browne RM, O'Loughlin EV, Bennet-Wood V, Bourke J, Henning P, Hogg GG, Knight J, Powell H, Redmond D. Nationwide study of haemolytic uremic syndrome: clinical, microbiological, and epidemiological features. *Arch. Dis. Child.* 2001; **85**: 125–131.
52. Parma, AE, Sanz ME, Blanco JE, Blanco J, Vinas MR, Blanco M, Padola NL, Etcheverria AI. Virulence genotypes and serotypes of verotoxigenic *Escherichia coli* isolated from cattle and foods in Argentina. *Eur. J. Epidemiol*. 2000; **16**: 757-762.
53. Johnson RP, Clarke RC, Wilson JB, Read SC, Rahn K, Renwick SA, Sandhu KA, Alves D, Karmali MA, Loir H, McEwen SA, Spika JS, Gyles CL. Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verotoxigenic *Escherichia coli*. *J. Food Prot.* 1996; **59**: 1112-1122.
54. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV Food-related illness and death in the United States. *Emerg. Infect. Dis*. 1999; **5**: 607-625.
55. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med*. 1983; **308**: 681-685.
56. Brett KN, Hornitzky MA, Bettelheim KA, Walker MJ, Djordjevic SP. Bovine Non-O157 Shiga Toxin 2-Containing *Escherichia coli* Isolates Commonly Possess stx2-EDL933 and/or stx2vhb Subtypes. *J. Cli Microbiol.* 2003; **41**(6): 2716-2722.
57. Wani SA, Pandit F, Samanta I, Bhat MA, Buchh AS. Molecular epidemiology of Shiga toxinproducing *Escherichia coli* in India. *Curr. Sci*, 2004; **87**(10): 1345-1353.
58. Kimmitt PT, Harwood CR, Barer MR. Induction of type 2 Shiga toxin synthesis in *E. coli* O157 by 4-quinolones. *Lancet* 1999; **353**: 1588-1589.
59. Kimmitt PT, Harwood CR, Barer MR. Toxin gene expression by shiga toxin-producing Escherichia coli: the role of antibiotics and the bacterial SOS response. *Emerg. Infect. Dis*. 2000; **6**: 458-465.
60. Zhang X, McDaniel AD, Wolf LE, Keusch GT, Waldor MK, Acheson DW. Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. *J. Infect. Dis.* 2000; **181**: 664-670.
61. Wong CS, Jelacic C, Habeeb RL, Watkins SL, Tarr PI. The risk of the haemolytic uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N Engl J Med* 2000; **342**: 1930-1936.
62. Guraya R., Frank J.F., Hassan A.N., (1998). Effectiveness of salt, pH, and diacetyl as inhibitors for Escherichia coli O157:H7 in dairy foods stored at refrigeration temperatures. *J. Food Prot*.1998; **61**: 1098-1102.
63. Yoichi M, Morita M, Mizoguchi K, Fischer CR, Unno H, Tanji Y. The criterion for selecting effective phage for *E. coli* O157:H7 control. *Biochem Eng J*., 2004; **19**(3): 221-227
64. Moradpoura, Z, Ghasemian, A. Modified phages: Novel antimicrobial agents to combat infectious diseases. *Biotech Adv*. Article in Press, Corrected Proof 2011.
65. Kelly BG, Vespermann A, Bolton DJ. Gene transfer events and their occurrence in selected environments. Food and Chemical Toxicology 2009; **47**: 978-983.
66. Masuda A, Yushikai Y, Aiba K, Matsuguchi T. T_H2 cytokine production from mast cells is directly induced by lipopolysaccharide and distinctly regulated by c-fun N-terminal kinase and p38 pathways. *J immunol.* 2002; **169**: 3801-3810.
67. Bonten MJM. Selective Digestive Tract Decontamination—Will It Prevent Infection with Multidrug-Resistant Gram-Negative Pathogens but Still Be Applicable in Institutions where Methicillin-Resistant *Staphylococcus aureus* and Vancomycin-Resistant Enterococci Are Endemic? *Clin Infect Dis.* 2006; **43** (Supplement 2): S70-S74.
68. Hermon-Taylor J. *Mycobacterium avium* subspecies paratuberculosis, Crohn's disease and the Doomsday Scenario. *Gut Pathogens*, 2009;

1:15 doi: 10. 1186/1757-4749-1-15

69. EFSA. Urgent advice on the public health risk of Shiga-toxin producing *E. coli* in fresh vegetables. *EFSA J*, 2011; **9**(6): 2274.
70. James, J. Overview of Microbial Hazards in Fresh Fruit and Vegetables Operations in: Overview of microbial hazards. Microbial Hazard Identification in Fresh Fruit and Vegetables, John Wiley & Sons, Inc. 2006; 1-36.
71. Arumugam, M., Raes, J., Pelletier, E., *et al.,* Enterotypes of the human gut microbiome. *Nature* 2011; **473**: 174-180.
73. Nataro JP, Kaper JB. Diarrheagenic *E coli*. *Clin. Microbiol. Rev,* 1998, **11**(1): 142-201.
72. Bhavsar AP, Guttman JA, Finlay BB. Manipulation of host-cell pathways by bacterial pathogens. *Nature*. 2007; **449**: 827-834
74. Deborah CH, Frankel G. Enteropathogenic *Escherichia coli*: unravelling pathogenesis. *FEMS Microbiol Rev*. 2005; **29**: 83-98.
75. Bommarius B, Maxwell D, Swimm A, Leung S, Corbett A, Bornmann W, Kalman D. Enteropathogenic *Escherichia coli* Tir is an SH2/3 ligand that recruits and activates tyrosine kinases required for pedestal formation. *Mol Microbiol*. 2007; **63**: 1748–1768.
77. Proulx F, Seidmann EG, Karpmann D. Pathogenesis of Shiga toxin -associated haemolytic uremic syndrome. *Pediatr. Res*. 2001; **50**:163-171.
78. Caprioli A, Morabito S, Brugere H, Oswald E. Enterohaemorrhagic *Escherichia coli:* emerging issues on virulence and modes of transmission. *Vet Res* 2005; **36**: 289-311.
79. Spangler BD. Structure and function of cholera toxin and the related *E. coli* heat-labile enterotoxin. *Microbiol Rev* 199256: 522-647.
80. Huang DB, Mohanty A, Dupont HL, Okhuysen PC, Chang T. A review of an emerging enteric pathogen: Enteroaggregative *E. coli*. *J Med Microbiol* 2006; **55**: 1303-1311.
81. Huang DB, Dupont HL, Jiang ZD, Carlin L, Okhuysen PC. Interleukin-8 response in an intestinal HCT-8 cell line infected with enteroaggregative and enterotoxigenic *E. coli*. *ClinDiagn Lab Immunol* 2004; **11**: 548-551.
82. Harrington SM, Straumann MC, Abe CM, Nataro JP. Aggregative adherence fimbrae contribute to the inflammatory response of epithelial cells infected with enteroaggregative *E. coli*. *Cell Microbiol* 2005; **7**: 1565-1578.
83. Nataro JP. Enteroaggregative *Escherichia coli* pathogenesis. *Curr. Opin. Gastroenterol*. 2005; **21**: 4-8.
84. Harrington SM, Dudley EG, Nataro JP. Pathogenesis of enteroaggregative *E. coli* infection. *FEMS Microbiol. Lett* 2006; **254**: 12-18.
85. Steiner TS, Nataro JP, Poteet-Smith CE, Smith JA, Guerrant RL. Enteroaggregative *Escherichia coli* expresses a novel flagellin that causes IL-8 release from intestinal epithelial cells. *J. Clin Invest* 2000; **105**:1769-1777.
86. Menard LP, Dubreiul JD. Enteroaggregative *Eshcherichia coli* heat stable enterotoxin 1 (EAST1): a new toxin with an old twist. *Crit. Rev. Microbiol*. 2002; **28**: 43-60.
87. Scheutz F, Moller Nielsen E, Frimodt-Moller J, Boisen N, Morabito S, Tozzoli R, Nataro JP, Caprioli A. Characteristics of the enteroaggregative Shiga toxin/verotoxin producing *Escherichia coli* O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. *Euro. Surveill*. 2011; **16**(24): 198-199.
88. Peiffer I, Bernet-Camard MF, Rousset M, Servin AL. Impairments in enzyme activity and biosynthesis of brush border-associated hydrolases in human intestinal Caco-2/TC7 cells infected by members of the Afa/Dr family of diffusely adhering *E. coli*. *Cell Microbiol*. 2001, **3**(5): 341-57.
89. Rich C, Alfidja A, Sirot J, Joly B, Forestier C. Identification of Human Enterovirulent *E. coli* Strains by Multiplex PCR. *J. Cli Lab Analysis* 2001; **15**:100-103.
90. Fujioka M, Kasai K, Miura T, Sato T, Otomo Y. Rapid Diagnostic Method for the Detection of Diarrheagenic *Escherichia coli* by Multiplex PCR. *Jpn J Infect Dis.*2009; **62**(6): 476-480.
91. Piercefield EW. Hemolytic Uremic Syndrome After an *Escherichia coli* O111 Outbreak. *Arch Intern Med*. 2010; **170**(18): 1656-1663.
92. Wang S. Rapid and Specific Detection of Enterotoxigenic *Escherichia coli* and Salmonella Strains by Multiplex PCR Systems. *J. Food and Drug An*, 2008; **16**(2): 81-87.
94. Dombek PE, Johnson LK, Zimmerley ST, Sadowsky MJ. Use of Repetitive DNA Sequences and the PCR To Differentiate *E. coli* Isolates from Human and Animal Sources. *Appl Envir Microbiol*. 2000; **66**(6): 2572-2577.
95. Bhan MK, Mahalanabis D, Fontaine O, Pierce NF. Clinical trials of improved oral rehydration salt formulations: a review. *Bull. World Health Organ*. 1994; **72**: 945-955.
96. Brussow H. Phage therapy: the *Escherichia coli* experience. *Microbiol.* 2005; **151**(7): 2133-2150.
97. Paton JC, Paton AW. Pathogenesis and diagnosis of shiga-toxin producing *Escherichia coli* infections. *Clin Microbiol Rev*. 1998; **11**: 450-479.

98. Takeda T, Tanimura M, Yoshino K, Matsuda E, Uchida H, Ikeda N. Early use of antibiotics for STEC O157 : H7 infection reduces the risk of hemolytic uremic syndrome. Presented at the 3rd International Symposium and Workshop on Shiga Toxin (Verotoxin)-Producing *Escherichia coli* Infections. Melville, NY: Lois Joy Galler Foundation for Hemolytic Uremic Syndrome. 2007.
99. Mora A, Blanco JE, Blanco M, Alonso MP, Dhabi G, Echeita A, Gonzalez EA, Bernardez MI, Blanco J. Antimicrobial resistance of Shiga toxin (verotoxin)-producing *E. coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. *Res Microbiol*. 2005; **156**(7):793-806.
100. Bell BP, Griffin PM, Lozano P, Christie DL, Kobayashi JM, Tarr PI, Predictors of haemolytic uremic syndrome during a large outbreak of *Escherichia coli* O157:H7 infections. *Paed* 1997; **100**: 127(E12).
101. Cimolai N, Morrison BJ, Carter JE. Risk factors for the central venous system manifestations of gastroenteritis associated haemolytic uremic syndrome. *Paed* 1992; **90**: 616-621.
102. Tarr PI, Gordon CA, Chandler WL. Shiga toxin producing *Escherichia coli* and haemolytic uremic syndrome. *Lancet* 2005; **365**: 1073-1086
103. Guarino A, Albano F, Ashkenazi S, Gendrel D, Hoekstra JH, Shamir R, Szajewska H. European society for paediatric infectious diseases/ European society for paediatric gastroenterology, hepatology and nutrition evidence-based guidelines for the management of acute gastroenteritis in children in *Europe. J. Pediatr. Gastroenterol. Nutri*. 2008; **46**: S1-S41
104. Adams M. *E. coli* Superbug Outbreak in Germany Due to Abuse of Antibiotics in Meat Production. Free from harms. July 19 edition: http://freefromharm.org/food/food-safety-food/e-coli-superbug-outbreak-in-germany-due-to-abuse-of- antibiotics-in-meat-production/ 2011.
105. CDG, Center for Global Development. *The race against drug resistance*. A report of the Center for Global Development's Drug Resistance Working Group. Washington, DC: Center for Global Development. 2010.
106. James Beard Foundation Awards, JBFA. Drug Resistant Superbugs Worse Than Anyone Could Imagine. http://blog.friendseat.com/drug-resistant-superbugs-getting-worse, downloaded on 19th July, 2011; **18**: 53.