Database Development of Primers for the Detection of Food Pathogens

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Submitted by

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CERTIFICATE

This is to certify that the M. Tech. dissertation entitled **"Database Development of Primers for the Detection of Food Pathogens."**, submitted by **BHANU CHOHLA (2K12/BIO/004)** in partial fulfilment of the requirement for the award of the degree of Master of Engineering, Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work carried out by her under my guidance.

The information and data enclosed in this dissertation is original and has not been submitted elsewhere for honouring of any other degree.

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Bhanu
(2K12/Bio/04)

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LIST OF ABBREVATIONS

DNA	Deoxyribo Nucleic Acid
mRNA	Messenger Ribo Nucleic Acid
mRNA	Messenger Ribo Nucleic Acid
rRNA	Ribosomal Ribo Nucleic Acid
BLAST	Basic Local Alignment Search Tool
CDS	Centres for Disease Control and Prevention.
CGI	Common Gateway Interface
HTML	Hyper Text Markup Language
NCBI	National Centre for Biotechnology Information
PCR	Polymerase Chain Reaction
PCR RT-PCR	Real Time-Polymerase Chain Reaction
PERL	Protein Extraction Report Language
UPL	Universal Probe Library
WHO	World Health Organisation

Database Development of Primers for the Detection of Food Pathogens.

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1.ABSTRACT

Background: According to WHO report about one-third of people in developed countries are affected by food-borne pathogens each year. In developing countries this figure is significantly higher. Globalization of the trade means that contaminated food has the potential to spread widely. Therefore food pathogen became a challenge for Global Public health.

Description: In order to detect Food pathogen, we have developed a user friendly comprehensive database to predict Insilco primer sequences (http.data base of food pathogen.bisr.res.in).There is an enormous number of microbial pathogen, we have studied mainly food pathogen responsible to cause foodborne illness. We have extracted information regarding their pathogenies, food source and their associated diseases and symptoms fallowed by them. Bacteria come out as most widely distributed food pathogen. Most of the time infections result in diarrhoea or dysentery, nausea followed by vomiting and abdominal cramping they have varying onset time. Myocotoxic or secondary metabolites are also responsible to cause food-borne illness. Protozoans are emerging as opportunistic food pathogen. Mainly the cases of food-borne illness are self-limiting and mild but occasionally they may lead to cause serious health hazard or may result in death.

Conclusions: This database is expected to discriminate species specific pathogen and give better insight to sense the relationship between contaminated foods and illness supports food safety along the entire food production chain

2. INTRODUCTION

2.1 Background

Food-borne pathogens are the main vehicle to cause food-borne illness. Mainly food-borne illness is associated with infection caused by microbial pathogens that have entered the food chain at some time by farm to fork. Pathogens can be unwittingly acquainted via a food source. There are an enormous number of microbial pathogen; Bacteria (*E. coli, Salmonella, Campylobacter, Clostridium, Bacillus cereus*), viruses *(Norwalk agent, Rotaviruses*), and parasites (*Giardia, Entamoeba histolytica, Cryptosporidium parvum*) that cause infection and food-borne illness. Most of the time infections result in diarrhoea or dysentery, nausea, vomiting, and abdominal cramping. Microbial toxin or Secondary metabolites are also responsible to cause food-borne illness. Mainly the cases of food-borne illness are self-limiting and mild but occasionally they may lead to cause serious health hazard or may result in death. According to **WHO report about one-third of people in developed countries are affected by food-borne pathogens each year**. In Developing countries this figure is significantly higher. Globalization of the trade means that contaminated food has the potential to spread widely. Therefore food-Pathogen became a challenge for Global Public health(Foodborne disease outbreaks: guidelines for investigation and control, 2008). Due to the increasing Outbreaks in food pathogen borne illness, the identification and eradication of the pathogens had gained interest, (GNF; Strategic Plan 2011-2015). The use of oligonucleotides had proved to be one of the best techniques available for the species detection in recent years. Bioinformatics tools and approaches such as NCBI (National Centre for Biotechnology Information), BLAST (Basic Local Alignment Search Tool), UPL (Universal Probe Library) , *UniProt* (Universal Protein Resource), Perl Scripting, CGI (Common Gateway Interface) allows us to develop high speed processor that will save time and coast to predict the specific primers and probes for the organisms with a good accuracy.

We have developed a comphernesive Database For Food Pathogens to predict Insilco primer sequences via using Universal Probe Library. We have mainly taken the list of the Major and emerging Pathogen according to W.H.O guideline(Appendix.1) and extracted information regarding pathogenic factor and pathogen associated food contaminants sources ,various symptoms and disease associated with Food Pathogen that are mainly responsible to cause infection and foodborne illness.

2.2 Aim and Objective:

This study mainly emphasis on Database Development of Primers for the Detection of Food Pathogens. Main objectives are:

1. To study Food-Pathogen.

2. Identify pathogenic Factor and probe.

3. Insilico primer designing .

3. REVIEW OF LITERATURE

The food we eat, water and the beverages we drink can get contaminated by bacteria, viruses, parasites, fungal toxin or chemicals that can cause food-borne disease or food poisoning. Most people some time in their lives have experienced at least intestinal upset by eating food or drinking a beverage that was improperly stored or prepared, insufficiently cooked, contaminated food**.** Food-related illness or food-borne disease is caused by a wide variety of pathogens and toxins. Because the microbe or toxin enters the body through the gastrointestinal tract, the most common symptoms of these illnesses are nausea, vomiting, diarrhoea and abdominal cramps. However, food-borne illness can vary dramatically in terms of how soon symptoms begin after eating or drinking the contaminated food, the length of illness, and when and how well a person recovers. Also, many germs or pathogens that can contaminate food items may be transmitted by other means, such as contact with infected animals, contact with ill persons, faeces or even as a result of laboratory accidents. **Each year 48 million people gets sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases** as estimated by **CDC 2011 estimate report.** CDC had estimates for two major groups of foodborne illnesses:

Known foodborne pathogens: 31 pathogens has been identified and proven as cause of foodborne illness by tracking public health systems to know concern diseases and outbreaks.

Unspecified agents: Agents are show their presence, but not under considered as cause of food illness due to lack of sufficient data.

3.1.Foodborne Illness:

Occurs when a pathogen is ingested with food and establishes itself (and usually multiplies) in the human host, or when toxigenic pathogens establishes itself in a food product and produces a preformed toxic microbial product is then ingested by the human host. In general, a foodborne illness outcomes can be caused by contamination of food via biological agents or pathogens (*e.g.,* viruses, bacteria, parasites, prions), chemical agents (*e.g.* Fungi: toxins, metals), or physical agents (*e.g.* glass fragments, bone chips). With the exception of certain parasites, nearly all foodborne pathogens are microscopic in nature. In increasing order of size, these pathogens include viruses, bacteria, protozoa and other parasites.

Thus, foodborne illness is generally classified into two main categories, **foodborne infection and foodborne intoxication,** as follows:

3.1.1. **Foodborne Infections:** occur as a consequence of growth of the pathogen in the human body. Since an incubation period is usually involved, the time from ingestion until symptoms occur is much longer than that of foodborne intoxications. The two basic categories of foodborne infections are:

3.1.2. **Invasive Infections:** which are caused by pathogens that invade body tissues and organ. Groups included in these groups are the viruses, parasitic protozoa, other parasites, and invasive bacteria(e.g., ***Salmonella, Aeromonas, Campylobacter, Shigella, Vibrio***

parahaemolyticus, ***Yersinia*** and **enteric-type** ***Escherichia coli***). **Toxicoinfections :** which are caused by infective bacteria that are not considered invasive in nature, but are capable of multiplication or colonization in human intestinal tract and produce toxins. Included in this group are: ***Vibrio cholerae, Bacillus cereus*** (diarrheal-type)**,** ***C. botulinum*** (in infants)**,** ***C. perfringens*** and verotoxigenic ***E. coli*** **(*E. coli* O157:H7** and others).

Infection from food-borne pathogens may be caused by bacteria, parasites or viruses. Common food-borne pathogens are *Escherichia coli* (*E. coli*), *Salmonella*, *Norovirus* and *Listeria*. Food-related illnesses from infectious pathogens typically take much longer to produce symptoms than toxins do. In the case of *E. coli* infections, symptoms may not appear until 10 days after a person has consumed the contaminated food item. Symptoms from these illnesses may also last longer than intoxication symptoms and are, in general, more easily passed from person to person (Ronald *et.al* 2012).

3.2.1: **Foodborne Intoxications:** The term "intoxication" or foodborne disease is the type of illnesses caused by toxins that may be in the food we eat. These toxins may be produced by bacteria growing on food that has not been handled properly; may result from chemicals, heavy metals and other substances in food; or because fish, shellfish or other animals have concentrated toxins in their flesh from their feeding habits and environment. In general, symptoms caused by toxins occur very soon after eating a contaminated food and may result in sudden and uncontrollable vomiting and/or diarrhoea. The primary bacteria causing foodborne intoxications include: ***C. botulinum, B. cereus*** (emetic-type) and ***Staphylococcus aureus*** (CDS: Alert, 2009). Other non-bacterial toxins that cause illness include:

• Paralytic shellfish toxin (caused by the consumption of mussels, clams and scallops which have ingested toxic dinoflagellates),

• Ciguatera toxins (associated with certain tropical fish),

• Scombroid toxins (results from the production of histamine due to bacteria spoilage of fish), and Fungal toxins or mycotoxins that can be of long-term carcinogenic concern with consumption of mold contaminated foods (e.g., aflatoxins in contaminated corn, peanuts, or other foods and patulin from contaminated apple or other fruit products).

Bacteria are resistance to high salt, sugar, or total solids level as well as the acidity of food products. Pathogens are capable of survival at low pH.(Rodrick ***et.al***.2003).Bacteria has the ability to produce toxins, intoxication is mostly caused by bacterial food contamination. Intoxication involves food poisoning in which the bacteria grows in food and releases a toxin.

	Foodborne infection	Foodborne disease
Cause	Bacteria / Viruses / Toxin	Toxin
Mechanism	Invade and / or multiply within the lining of the intestines.	No invasion or multiplication
Incubation period	Hours to days	Vomiting, Nausea, Double vision Minutes to hours
Symptoms	Diarrhoea Nausea / Vomiting, Nausea, Abdominal cramps ± Fever	Double vision, Weakness Respiratory failure Numbness Sensory and motor dysfunction
Transmission	Can spread from person-to-person via the faeco-oral route	Not communicable
Factors related to food contamination	Inadequate cooking Inadequate cooking ,cross –contamination ,poor personal hygiene	Inadequate cooking Inadequate cooking, improper holding temperature

Table 3.1 Foodborne infection versus foodborne Disease

Usually immunochomphrised, very young, elderly persons are more prone to get infected. Anyone can become ill from eating contaminated food items. Every year in the United States, 17 percent of Americans get sick as a result of consuming contaminated foods or beverages.

3.3 **Classification of Foodborne Causative Agents:**

Bacteria are one-celled microorganisms with a cell wall but no nucleus. They exist in a variety of shapes, types and properties. Some pathogenic bacteria are capable of spore formation and thus highly heat-resistant *(e.g., Clostridium botulinum, C. perfringens, Bacillus subtilus, B. cereus*). Others are capable of producing heat-resistant toxins (e.g., *Staphylococcus aureus*). Most pathogens are mesophilic with optimal growth temperature range from 20 to 45^0C (68 to 113^0F). However, certain foodborne pathogens (termed psychrotrophs) are capable of growth under refrigerated conditions or temperatures less than 10^0C (50^0F). The most well documented psychotropic foodborne pathogens are Listeria monocytogenes, and Yersinia enterocolitica. Listeria monocytogenes, for example, will grow (albeit slowly) at temperatures just of above freezing (approximately 33-34^0F). Certain strains or serotypes of Bacillus cereus, Clostridium botulinum, Salmonella spp., E. coli O157:H7, and Staphylococcus aureus may also grow slowly under refrigeration conditions. Bacteria also vary in (Ronald *et.al* 2012).

Endotoxin are cell associated structural components; lipopolysaccharides while Exotoxin are usually secreted by bacteria. Exotoxins are usually proteins, minimally polypeptides. Bacterial enterotoxin mainly infect Proximal small intestine with non-inflammatory action followed by watery diarrhoea while bacterial cytotoxic effect colon and distal small intestine fallowed by Dysentery /inflammatory diarrhoea cytotoxic invasion show inflammatory evidence.Batcterial contaminated food source, such as sugars, starch, protein, fats and other

compounds provide the nutrients. Outbreaks caused by bacterial pathogens were particularly severe. For example, Listeria outbreaks resulted in the highest proportion of persons hospitalized (82%), followed by Clostridium botulinum (67%).

3.3.1. **Heat Stable Enterotoxins**

Heat-stable enterotoxins (ST) are a family of conserved peptides expressed by pathogenic strains of Escherichia coli. ST elicits fluid accumulation in the intestine, which is often responsible for diarrhoea in travellers, young children, and domesticated animals in developing countries. ST includes two subfamilies, STa and STb. The STa subfamily that intoxicate humans (STah) (James et.al 2010).

3.3.2 Pore forming toxins

Bacterial pore-forming toxins (PFT) are a large group of protein toxins which forms pores in the membranes of bacteria, plants, and mammals, causing membrane permeability and ion imbalance

3.3.4 S**uperantigen-like toxins (SSL)**

Superantigens (SAgs) are a group of secreted protein toxins produced by an increasing numbers of bacteria, including *Staphylococcus aureus*, *Streptococcus sp*., Mycoplasma arithiditis, and *Yersinia spp*. SAgs bind major histocompatibility complex II (MHC II) and stimulate peptide-independent MHC II/T cell receptor (TCR) interaction and immune activation. SAgs is responsible for Toxic Shock Syndrome (TSS) and food poisoning.

3.3.5 Secretion of Toxins from the Bacterium

Bacterial toxins are transported across the bacterial membranes through co-translational and post-translational mechanisms to reach their targets. Toxin transport occurs by multiple mechanisms, which have been characterized within Gram Negative and Gram Positive bacteria. Most secretion systems utilize active transport, requiring at least one energy requiring step. (Mc Cormick ***et.al*** 2001).

3.3.6 Botulinum Neurotoxin Subtypes

Each BoNT serotype comprises subtypes that can vary between 3 and 32% at the primary amino acid level. There are seven types of *C. botulinum*, A, B, C, D, E, F and G, based on the serological specificity of the neurotoxin produced. Food-borne botulism is associated with types A, B, E and very rarely F. (James ***et.al*** 2010).

	Location Illness	Illness	Examples
Non-inflammatory (enterotoxin)	Proximal small intestine	Watery diarrhoea	*Vibrio cholerae*, ETEC, EAggEC, Cl. (enterotoxin) small diarrhoea leukocytes *perfringens, Bacillus cereus, Staph aureus*
Inflammatory invasion (cytotoxin)	Colon / Distal small intestine	Dysentery /inflammatory	*Shigella, Salmonella, C. jejuni, EHEC, enterocolitica, Vibrio parahaemolyticus, Cl. difficile, E. histolytica*
Penetrating	Distal small intestine	Enteric fever	*Salmonella typhi, Y. enterocolitica, Campylobacter fetus*

Table: 3.2 Categorised the bacterial pathogen on the basis of mechanism followed by them to cause illness, associated with infected location.

I.P.	Cause	Symptoms	Common foods
1-6 hours	*Staph aureus*	Nausea, Vomiting, Diarrhoea	Ham, poultry, potato / egg salad, mayonnaise, cream pastries
	Bacillus cereus	Nausea, Vomiting, Diarrhoea	Fried rice
8-16 hours	*Cl. perfringens*	Abdominal cramps, diarrhoea (vomiting rare)	Beef, poultry, legumes, gravies
	B. cereus		Meats, vegetables, dried beans, cereals
>16 hours	*Vibrio cholerae*	Watery diarrhoea	Shell-fish
	ETEC	Watery diarrhoea	Salad, cheese, meats, water
	EHEC	Bloody diarrhoea	Beef, salami, raw milk / vegetables, apple juice
	Salmonella sp	Inflammatory diarrhoea	Beef, poultry, eggs, diary products
	Campylobacter jejuni	Inflammatory diarrhoea	Poultry, raw milk
	Shigella sp	Dysentery	Potato / egg salad, lettuce, raw eggs
	V. parahaemolyticus	Dysentery	Molluscs, crustaceans

Figure: 3.1. Representing bacteria contaminated food source along with their incubation period and associated symptom's (CD: Alert 2009)

3.4 Viruses are particulate in nature and multiply only in other living cells. Thus, they are incapable of survival for long periods outside the host. An infected cell then starts making more viruses until it can't make any more, breaking open and releasing the new viruses into the body to infect more cells. Viral gastroenteritis is usually regarded as a mild self-limiting disease lasting 24-48 hours. However, people can feel debilitated for 2 to 3 weeks, fallowed

Wide range of symptoms malaise, abdominal pain, pyrexia, diarrhoea and/or vomiting .occurring in an outbreak should alert investigations to the possibility of a viral cause .While greater than 100 types of enteric viruses have been shown to cause foodborne illness, the most common foodborne virus pathogens are:

Time to onset of symptom	**Associated Symptoms/** Illness	Mechanism	Location	organism
12–48 (median 36 h)	Nausea, vomiting, watery non-bloody diarrhoea, dehydration	Non-inflammatory (enterotoxin)	Proximal small intestine	Norovirus
3-5 days	Fever, vomiting, watery non-inflammatory diarrhoea	Non-inflammatory (enterotoxin)	Proximal small intestine	Rotavirus, Astrovirus Enteric adenoviruses

Table.3.3: Representing Viral pathogen along with their incubation period and associated symptom's(WHO Guidelines 2008; CD: Alert 2009).

3.4.1 ***Norovirus***: Is emerging pathogenic calicivirus, recognized as the leading cause of gastroenteritis and of food-related outbreaks (.CDC: estimated sheet 2014) .At least 34 genotypes and 5 gene groups are known. Primarily by GI and GII and during the past two decades most outbreaks in the industrialized world were due to variants of genotype GII.4. New GII.4 variants have emerged every 2–3 years, replacing previously predominant GII.4 strains and often resulting in increased outbreak activity (Sabine *et al*. 2013).

3.4.2. ***Rotaviruses***: Mainly infect young children. It is estimated that they causes one million deaths a year in children under 5 years of age, mostly in developing countries. In developed countries deaths are relatively rare, but rotavirus gastroenteritis is the most frequent reason for admission of young children to hospital. Rotaviruses consistently account for around 80% of all gastroenteritis viruses. Food-borne and particularly water-borne spreading are probably a significant route of transmission in developing countries, but in developed countries reports are rare.

3.4.3. ***Hepatitis A***: Is referred to as one of the oldest diseases known to humankind by the World Health Organization (WHO)**.** The term hepatitis refers to having inflammation of the liver. This inflammation can be caused by any strain of hepatitis. These strains include hepatitis A, B, C, D or E. Virulence factors associated with HAV include viral agents that produce an immune response.HAV has been called epidemic hepatitis generally self-limited and can produce effects that range from a lack of symptoms to death HAV is primarily hepatotoxic; it replicates in the liver, produces a viremia. The World Health Organization estimated paradoxically, hepatitis A virus could re-emerge in regions where it is not endemic. Risk for outbreaks with more severe illness becomes greater in countries where such epidemiologic transition has occurred. In countries with low levels of HAV, the main risk comes from travel, secondary waves of transmission.(However, the probable source of infection remains unknown for 20%–30% of cases, possibly because of transmission by persons with subclinical or missed primary cases, but alternatively because of food contamination. Although HAV is listed as the second most common foodborne virus

Foodborne HAV infections are rarely reported, except when triggered by an unusual outbreak. Recently MSM strain recently reported in Netherlands restaurant. (Mariska ***et.al*** 2014).

3.5. Parasitic Protozoa: Are one-celled microorganisms without a rigid cell wall, but with an organized nucleus. They are larger than bacteria. Like viruses, they do not multiple in foods, only in hosts. The transmissible form of these organisms is termed a ***cyst***. Protozoa that have been associated with food and water-borne infections include *Entamoeba histolytica, Toxoplasma gondii, Giardia lambia, Cryptosporidium parvum* and *Cyclospora cayatenensis*.

3.5.2. ***Cyclospora cayatenensis***: Cyclosporiasis is an intestinal illness caused by the microscopic intercellular protozoan parasite. The organism has a complex life cycle that can take place in a single animal host. It produces oocysts (diameter 4-6 μm) which are very resistant to chlorination but killed by conventional cooking procedures. Associated Foods contaminant involve raw milk, drinking-water and apple cider. Worldwide. Cryptosporidiosis is one of the leading causes of diarrhoeal disease in infants and young children, accounting for 5–15 % of diarrhoeal disease cases in children (Maha ***et.al*** 2013;WHO guidelines 2008).

3.5.2. ***Toxoplasma*** **parasite:** In United states Toxoplasmosis is the second leading cause of death attributed to foodborne illness in the United States. More than 60 million men, women, and children carry the *Toxoplasma* parasite. However, women mainly infected with *Toxoplasma* during pregnancy and anyone with a compromised immune system should be aware that toxoplasmosis can have severe consequences. Toxoplasmosis is considered one of the Neglected Parasitic Infections, a group of five parasitic diseases that have been targeted by CDC for public health action

Time to onset of symptom	Characteristic agent	Illness/symptoms	Location	Organism
2-4 weeks (range several days to several months).	Cysts	Amoebic dysentery/ Sever bloody diarrhoea, stomach pains, fever and vomiting. Most infections remain	Gastrointestinal tract	*Entamoeba histolitica*
2-4 days. (range from several days to weeks)	oocysts	Cryptosporidiosis/ Persistent diarrhoea, nausea, vomiting and abdominal pain, sometimes accompanied by an influenza-like illness with fever.	Gastrointestinal tract	*Cryptosporidium parvum* (lowa II)
1-6 weeks	trophozoite	Diarrhoea, malabsorption/ Mucoid diarrhoea (fatty stools) abdominal pain, flatulence, weight loss	Gastrointestinal tract	*Giardia lamblia*

Table:3.4. Representing parasitic protozoan along with their incubation period and associated symptom's (WHO Guidelines 2008)

Multi-cellular Parasites are animals that live at the expense of the host. They may occur in foods in the form of eggs, larvae, or other immature forms. Trichinosis has been an important reportable pathogen associated with undercooked pork. Other parasites of concern include flatworms or nematodes (associated with fish), cestodes or tapeworms (usually associated with beef, pork, or fish) and trematodes or flukes (more or a concern outside the US)

3.6. Fungal Myocotoxic: metabolites known for their potent carcinogenic properties. The ability of aflatoxin production has been reported in various species of the *Aspergillus* genus. There are a number of teleomorphic (ascosporic) genera which have *Aspergillus* conidial states (anamorphs), but the only two of real importance in foods are the xerophilic genus *Eurotium* and *Neosartorya* which produce heat-resistant ascospores and cause spoilage in heat-processed foods (Nisreen Al-Hmoud ***et.al.,***(2012).Almost 50 species of *Aspergillus* have been identified as capable of producing toxic metabolites. Chief toxins produced by *Aspergillus* sp., are the aflatoxins, (*A. flavus, A. parasiticus, A. nomius*), Ochratoxin A (*A.*

ochraceus), Sterigmatocystin (*A. versicolor*), Cyclopiazonic acid (*A. flavus, A. tamari*), Citrinin, Patulin and Penicillic acids.

The majority of important toxigenic and food spoilage species are found in subgenus *Penicillium.* Growth of mould does not always mean production of toxin. The conditions under which toxins are produced are often narrower than the conditions for growth. Most toxins can be placed in two broad groups: those that affect liver and kidney function and those that are neurotoxins. The *Penicillium* toxins which affect liver or kidney function are asymptomatic.(Hema Ramanathan ,**2010**).

3.7..**Food-borne illness: signs and symptoms**: Usually, people suffering from a food-borne disease will have a combination of nausea, vomiting and diarrhoea and/or fever. Some pathogen haves lead to cause wide range symptoms, while others may show one or may be asymptomatic. Typically asymptomatic, food-borne illness only lasts a few days; however, sometimes more serious illness can occur. Persons may need to be hospitalized or may even die from a food-borne disease

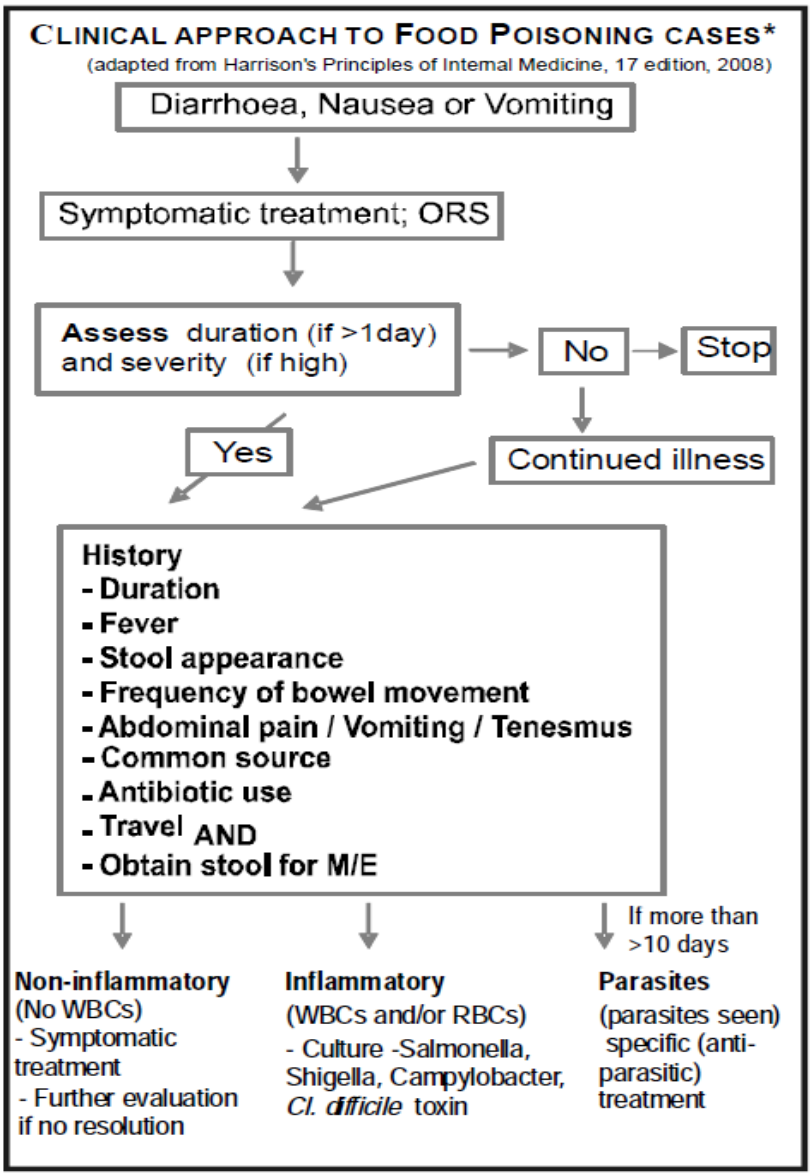

Figure 3.2.Clinical Approach to Food Poisoning (Harrison.2008)

3.8. Pathogenesis: Food-borne illness is typically caused by microorganisms or their toxins, and most often manifests with gastro-intestinal symptoms, which can vary in severity and duration. In addition to food-borne pathogens (bacteria, viruses and parasites), food-borne disease may also be caused by contaminants like heavy metals, chemicals, pesticides and toxic substances present naturally in food like toxic mushrooms, plants, fish or shellfish. The food-borne diseases due to infectious causes form the majority of cases, and are largely dependent on the inoculum size or the infective dose of the pathogen. This may be as small as 10 to 100 bacteria or cysts for *Shigella, Entero- Haemorrhagic E. coli (EHEC), Giardia lamblia* and *Entamoeba histolytica*, requiring minor lapses in hygiene for the faeco-oral transmission. The infective dose for *Vibrio cholerae* on the other hand is usually 105 – 108, and may be variable for Salmonella spp.(Fidelma ***et.al*** 2008).Many pathogens are harmful to humans exist naturally in much of the food we eat, such as meat and poultry, uncooked or

undercooked food, raw and pasteurised milk.. Usually, these pathogens are destroyed when the food is cooked. However, if the food is eaten undercooked or raw, or the food is handled improperly during preparation or storage, the risk for transmitting harmful pathogens to humans increases. Most of the Pathogens are ubiquitous in all habitats and are recognized by their metabolic versatility and ability to produce Pathogenic Factor Which may include, Toxins, Secondary Metabolites, Virulent strains which may leads to contaminate food sources and finally lead to cause Foodborne diseases. The term Food-borne diseases, including foodborne intoxications and food-borne infections, covers illnesses acquired through consumption of contaminated food, and are also frequently referred to as food poisoning. Toxin play lead role in foodborne illness. Toxins are any poisonous substance produced by a living organism that is capable of causing infection or disease or death in other organisms. Most of the pathogen can produce more than one toxin at the time (Tenreiro *et.al*, 2010). (Appendix 2, 3, 4)

- Invading and multiplying in the lining of the intestines and/or other tissues.(bacterial toxin ,parasites ,viruses).
- Invading and multiplying in the intestinal tract and releasing a toxin (mostly bacteria only).Food-borne illness is typically caused by microbial pathogens or their toxins, and most often food borne illness fallowed by gastro-intestinal symptoms, which can vary in severity and duration and sometime asymptomatc.In addition to food-borne pathogens (bacteria, viruses and parasites), food-borne disease may also be caused by other contaminants like heavy metals, chemicals, pesticides and toxic substances present naturally in food like toxic mushrooms, plants, fish or shellfish.

FOOD-BORNE TRANSMISSION OF PATHOGENS AND TOXINS:

Food may become contaminated during production and processing or during food preparation and handling

3.8.1. Food production and processing: Foods, such as fruits and vegetables, may be contaminated if washed or irrigated with water that is contaminated with pathogens from animal or human faeces. Animals naturally harbour many food-borne bacteria in their intestines that can cause illness in humans, but often do not cause illness in the animals. During slaughter, meat and poultry carcasses can become contaminated if they are exposed to small amounts of intestinal contents.

3.8.2. Food preparation and handling: Infected individuals - Most food-borne pathogens are shed in the faeces of infected persons and these pathogens may be transferred to others through food via the faecal-oral route. Bacteria present in infected lesions and normal nasal flora may also be transmitted from an infected food-handler to ready-to-eat foods.

3.8.3. Cross-contamination: Pathogens naturally present in one food may be transferred to other foods during food preparation if same cooking equipment and utensils are used without washing and disinfecting in between, especially in case of ready-to-eat foods. Inadequate

cooking temperature – With insufficient cooking bacteria can multiply and produce toxins within the food. Many bacterial toxins are heat stable and may not be destroyed by cooking.

3.9.1. Improper storage: Food held or stored at warm (10-50ºC) temperature allows multiplication of pathogens and is an important cause of foodborne outbreaks.(Hema Ramanathan.2010)

The greatest association with foods is with seafood .Ready to eat foods and preserved food, the USDA's Food Safety and Inspection Service (UFSIS) has declared E. coli O157:H7 is widely distributed in various food sources and specially accounts in raw ground beef. *E. coli* O157 remains a recognised and important human pathogen and a high priority for FSA action. Although incidence of human infections has fluctuated, there has been no overall trend in incidence (either up or down) since 2000. In comparison to *Campylobacter* and *Salmonella* cases are relatively rare but infections can result in serious conditions that may affect the blood, kidneys or nervous system and can be fatal, particularly in infants, young children and the elderly. It has been the cause of a number of large and serious foodborne outbreaks. (Sharon *et.al*.2013)The incidence of *Salmonella* cases has declined consistently since 2000. A number of National Control Programmes are currently in place for the control of *Salmonella* in eggs and poultry.

Viruses are an important cause of infectious intestinal disease and a proportion of these cases are due to foodborne transmission. March 2010, 55 incidents were reported to the FSA involving illness associated with raw oyster consumption that were believed to be due to Norovirus. Many cases are also thought to result from the introduction of viruses into food by infected food handlers. (adapted site :food.gov.uk)

Food safety practices have vastly improved in the processing environment as a result of these regulatory actions, as evidenced by the decreasing rates of infection by both Listeria and E. coli O157:H7. However, of the 121 foodborne outbreaks reported through FoodNet in 2011, almost half (49%) of the reported cases were attributed to noroviruses, and the number of Vibrio infections is on the rise. Most commonly food contaminated found on raw foods of many types unpasteurized milk and milk product, meat and meat product. The greatest association with foods is with seafood .Ready to eat foods and preserved food therefore unfit for human consumption.(**CD: Alert 2009**).

3.9. Foodborne Outbreaks

Food-borne diseases are a major global concern for public health, health burden leading to high morbidity and mortality. The global burden of infectious diarrhoea involves 3-5 billion cases and nearly 1.8 million deaths annually, mainly in young children, caused by contaminated food and water. According to the CDC, an estimated 76 million cases of food-borne disease are reported annually in the United States with approximately 5000 deaths (John *et.al* 2013).

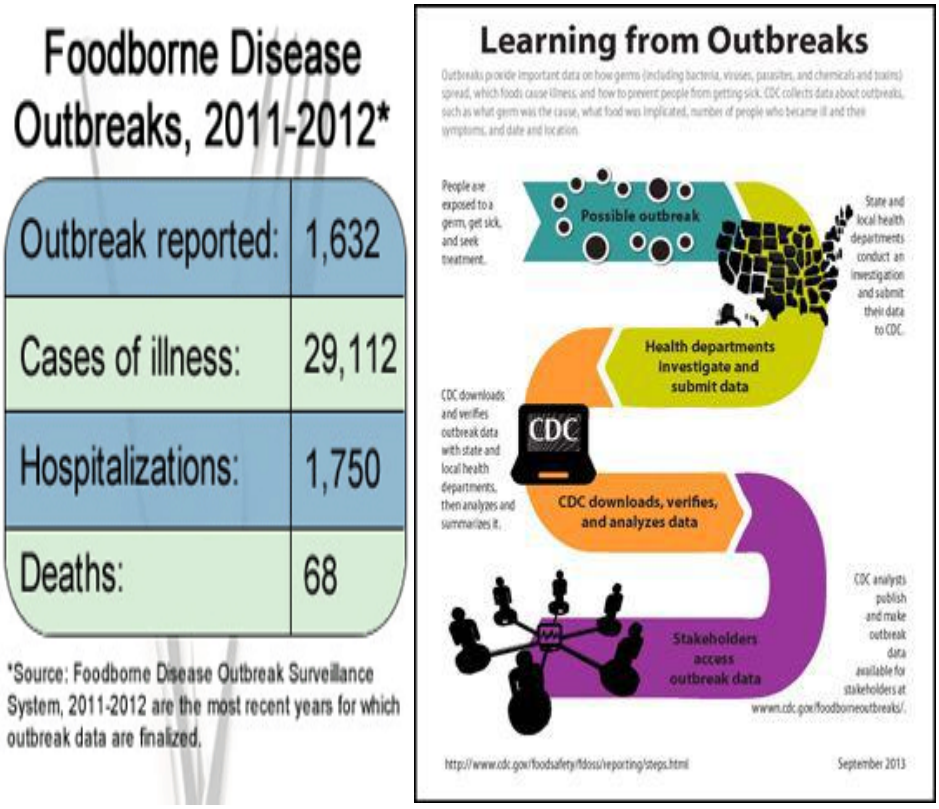

Figure 3.3. Foodborne disease outbreaks from 2011-2012

CDC's Foodborne Disease Outbreak Surveillance System gathers data on foodborne disease outbreaks from state, local, territorial, and tribal health departments (John *et.al.*, 2013).Data on Foodborne Disease Outbreaks: Associated food pathogens :During January 1, 2009 through December 31, 2010, public health departments reported 1,527 foodborne disease outbreaks, resulting in 29,444 cases of illness, 1,184 hospitalizations, and 23 deaths.

Table 1. Estimated annual number of domestically acquired foodborne illnesses, hospitalizations, and deaths due to 31 pathogens and unspecified agents transmitted through food, United States

Foodborne agents	Estimated annual number of **illnesses** (90% credible interval)	%	Estimated annual number of **hospitalizations** (90% credible interval)	%	Estimated annual number of **deaths** (90% credible interval)	%
31 known pathogens	9.4 million (6.6–12.7 million)	20	55,961 (39,534–75,741)	44	1,351 (712–2,268)	44
Unspecified agents	38.4 million (19.8–61.2 million)	80	71,878 (9,924–157,340)	56	1,686 (369–3,338)	56
Total	47.8 million (28.7–71.1 million)	100	127,839 (62,529–215,562)	100	3,037 (1,492–4,983)	100

National Center for Emerging & Zoonotic Infectious Diseases
Division of Foodborne, Waterborne, and Environmental Diseases

In 2013, a total of 19,056 infections, 4,200 hospitalizations, and 80 deaths were reported. For most infections, incidence was well above national Healthy People 2020 incidence targets and highest among children aged <5 years. Compared with 2010–2012, the estimated incidence of infection in 2013 was lower for *Salmonella*, higher for *Vibrio*, and unchanged overall. Since 2006–2008, the overall incidence has not changed significantly.

.

Eight known pathogens account for the vast majority of illnesses, hospitalizations, and deaths. Tables 2–4 list the top five pathogens causing illness, hospitalization, and death.

Table 2. Top five pathogens causing domestically acquired foodborne illnesses

Pathogen	Estimated annual number of illnesses	90% Credible Interval	%
Norovirus	5,461,731	3,227,078–8,309,480	58
Salmonella, nontyphoidal	1,027,561	644,786–1,679,667	11
Clostridium perfringens	965,958	192,316–2,483,309	10
Campylobacter spp.	845,024	337,031–1,611,083	9
Staphylococcus aureus	241,148	72,341–529,417	3
Subtotal			91

Table 3. Top five pathogens causing domestically acquired foodborne illnesses resulting in hospitalization

Pathogen	Estimated annual number of hospitalizations	90% Credible Interval	%
Salmonella, nontyphoidal	19,336	8,545–37,490	35
Norovirus	14,663	8,097–23,323	26
Campylobacter spp.	8,463	4,300–15,227	15
Toxoplasma gondii	4,428	3,060–7,146	8
E. coli (STEC) O157	2,138	549–4,614	4
Subtotal			88

Table 4. Top five pathogens causing domestically acquired foodborne illnesses resulting in death

Pathogen	Estimated annual number of deaths	90% Credible Interval	%
Salmonella, nontyphoidal	378	0–1,011	28
Toxoplasma gondii	327	200–482	24
Listeria monocytogenes	255	0–733	19
Norovirus	149	84–237	11
Campylobacter spp.	76	0–332	6
Subtotal			88

Figure.3.3. CDC : Fact sheet 2013 -2014.

- Among the 790 outbreaks with a laboratory-confirmed illness, norovirus was the most commonly reported infection, accounting for 42% of outbreaks; followed by *Salmonella*, with 30% of outbreaks. Out of 29,444 outbreak-related illnesses, 1,184 (4%) resulted in hospitalization. *Salmonella* caused the most outbreak-related hospitalizations (49%), followed by Shiga toxin-producing *E. coli* (16%), and norovirus (9%).

- Outbreaks caused by some pathogens were particularly severe. For example, *Listeria* outbreaks resulted in the highest proportion of persons hospitalized (82%), followed by *Clostridium botulinum* (67%), and paralytic shellfish poisoning (67%). Among the 23 deaths, 22 were linked to bacteria (9 *Listeria*, 5 *Salmonella*, *E. coli* O157, 3 *Clostridium perfringens*, and 1 *Shigella*), and 1 was linked to Norovirus (CDC:factsheet;2014).

- Thirty-eight multistate outbreaks were reported (16 in 2009; 22 in 2010). Twenty-one were caused by *Salmonella*, 15 by Shiga toxin-producing *E. coli* (thirteen O157, one O145, one O26), and two by *Listeria*. The pathogen was isolated from an contaminated food *Salmonella* (alfalfa sprouts ground turkey, shell eggs, frozen meal) and 6 caused by Shiga toxin-producing *E. coli* (ground beef, unpasteurized Gouda cheese, multiple unpasteurized cheeses, hazelnuts, and cookie dough).(James et.al 2010).

- *Y. pseudotuberculosis*, *Vibrio fluvialis* ,*norovirus* are the emerging pathogen . *Vibrio fluvialis* sole infected 80% patients with diarrhoea, Kolkata, India, 2002–2009(CD: Alertnewsletter 2009,India). Among speculated *Vibrio* isolates, the majority were *Vibrio parahaemolyticus* (62%).

- Mycotoxins are produced by certain Fungal or moulds that grow on various foods, including cereals, nuts and dried fruits. Mycotoxins are responsible to cause wide range of negative health effects in humans; some, such as aflotoxins, are carcinogenic in animals, and probably humans. Fungal Mycotoxins are predominant in India include alfatoxins, fumonisins, trichothecenes, ergot alkaloids and ochratoxins are predominate cause of rice and spices contamination.(Mohamed E. Zain;2011).

- The worldwide incidence of parasitic protozoan's and virus has been increasing; paralytic shellfish poisoning (PSP) estimated a 600 cases per year with a possible 300 of these being fatal. Outbreaks have been occurring regularly throughout the world.(John ***et al.,2013***).

Under the Integrated Disease Surveillance Project (IDSP) in India, food poisoning outbreaks reported from all over India in 2009 increased to more than double as compared to the previous year (120 outbreaks in 2009, as compared to 50 in the year 2008. This could be due to improved reporting. It is important to keep in mind that these are only the reported outbreaks and actual number of outbreaks may be much higher, since all cases or outbreaks do not get reported Therefore the food pathogens are the a real threat to human health globally.

4. MATERIALS AND METHODS

4.1 NCBI

NCBI (National Centre for Biotechnology Information) is a primary database of nucleotide and protein sequences. It stores information regarding to these sequences in both genbank (for nucleotide), genpept (for protein) and fasta format. We used this database in extraction of ;

- Genomes Information.
- Gene Identification

4.2 BLAST

BLAST (Basic Local Alignment Search Tool) is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA/RNA sequences.

tblastn: In case, nucleotide sequence of interest gene for a pathogen is not available, then this sequence is got from the result of tblastn i.e. used for finding similarity of protein sequence against to translated nucleotide sequences.

blastn: a **nucleotide** database searching using a **nucleotide** query. To validate Insilco primer result specificity.

4.3 UPL

In Universal Probe Library (UPL) there are 165 specific and pre-validated hydrolysis/Taqman probes that can quantify virtually any transcript of a large number of organisms. Universal Probe Library Gene Assays quantify expression levels of gene of interest in relation to an endogenous reference gene in a dual-color assay. Primer pairs designed by Probe Finder are checked by Insilco PCR. The algorithm searches the relevant genome and transcriptome for possible mis-priming sites for either of the two primer pairs. If any of the identified mis-priming sites are positioned in the genome or the transcriptome in a way that could potentially give rise to an unintended amplicon, the assay is down-graded and flagged as having failed the Insilico PCR check.

4.5 Steps used in project

STEP 1: Selection of Organisms

STEP 2: Preparation of the dataset

STEP 3: Extraction of Genome and pathogenic gene

STEP 4: Preparation of database of organisms with their genome.

STEP 5: Probe Designing

STEP 6: Finding species specific probes & primer

STEP 7: Preparation of database of species specific probes & primers.

STEP 8: Wet lab validation.

STEP 9: Web Implementation.

4.5.1 STEP 1: Selection of Pathogen: Food-borne pathogens are the main vehicle to cause food-borne illness. Mainly food-borne illness is associated with infection caused by microbial pathogens that have entered the food chain. According to WHO report about one-third of people in developed countries are affected by food-borne pathogens each year. Therefore food-borne illness became a challenge for Global Public health. Most of the pathogen we have selected on the basis of their severity and recently identified as cause of foodborne illness by considering WHO Guideline and CDC estimated report 2014.

4.5.2 STEP2: Preparation of the dataset: Extracted information regarding their genome information, concern Food source, about pathogenic factor responsible to cause disease and symptoms fallowed by them to cause disease.

Bacteria Pathogens	Fungai	Protozoa	virues
Bacillus cereus	*Aspergillus flavus*	*Cryptosporidium parvum*	*Hepatitis A virus*
Clostridium botulinum	*A. parasiticus.*	*Entamoeba histolytica**	*Hepatitis E virus*
Brucella melitensis	*Aspergillus ochraceus*	*Giardia lamblia*	*Norovirus**
Campylobacter jejuni*	*Aspergillus versicolor*	*Toxoplasma gondii**	*Poliovirus*
*E. coli**	*Aspergillus fumigatus*	*Cyclospora cayetanensis*	*Rotavirus A*
Listeria monoctyogenes*	*Aspergillus terreus*	*Nematoda*	*Astrovirus*
Salmonella Enteritidis Serovar	*Aspergillus clavatus*	*Anisakis simplex*	*Sapovirus*

*Staphylococcus aurenus**	*Eurotium repen*	*Trichinella spiralis*	*Adenoviruses E*
Streptococcus Pyogenes	*Penicillium expansum*		*Hepatitis E virus*
vibrio parahaemolyticus	*Penicillium citreonigrum*		
Vibrio vulnificus	*P. citrinum*		

Table 4.1. List of Foodborne Pathogens. (*Pathogen are emerge out as top pathogens as estimated by CDC report 2012-2014).

An example of each type of organism with its pathogenicity has been given in below table.

Pathogen	Pathological Information	Disease information	Food source
Bacteria: Bacilluscereus 3-4µm, Gram-positive, Rod-shaped (GI:196031951) cDNA (5269030 bp)	Cytotoxin K (CytK) : Associated with sever outbreak of Foodborn illness. PoreformingToxin ,responsible for food associated Diarrhoeal outbreak.	Diarrhoeal syndrome UrinaryTract Infections Puerperal sepsis Pulmonar Disease.	Milk, Dairy products, Vegetable dishes, Sauces
Virus:Norovirus Small,round, (GI:-106060735) (+) ssRNA, 7654bp	single genocluster, the GII.4 noroviruses, currently accounts for approximately 80% of all infections.	Gastrointestinal diseases	Composite meat, soup, chicken with curry, meat stew, mashed potatoes, sandwiches
Toxoplasma gondii, 4.7X2.1um. ,ovoid Zoonoticparasit (GI:559184048) 870956 rcDNA	Food-borne contamination mainly associated with tachyzoites. Therefore, the horizontal transmission of Toxoplasma via tachyzoites . Type I isolates are highly virulent, inoculation of <10 tachyzoites, while type II or III strains are considered avirulent strains,	Life-threatening disease;e.g. Encephalitis, Retinitis,Myocarditis in developing fetuses and in immune	By eating raw or undercooked, contaminated meats like pork lamb, or wild game and beef, but also

	allowing survival after the inoculation of >103 tachyzoites	compromised patients.Mainly responsible to cause illness.	seafood's
Aspergillum flavus	A. flavus produces aflatoxins and B 1 and B 2 and cyclopiazonic acid. These toxins are usually found together in various foods and feeds in various Major toxin aflatoxins of concern are designated B1, B2, G1, and G2.	cute liver damage; liver cirrhosis; induction of tumours; and teratogenic effects	Soft Cheeses Hard Cheeses Fish and Fish Products Fruits and Vegetables

Table 4.2 – Pathogens associated information about pathogenicity, infected food source

4.5.3 STEP 3: Extraction of Genome:

NCBI database has been used to extract genome and gene information if available. If not then switch to available protein sequence ,we extracted available protein translated in nucleotide sequence by using BLAST Tool (**tblastn).** (Appendix2,3,4.)

C	D	E	F	G
Classification	shape and size	GENOME INFORMATION	About	pathplogical information
Domain: Bacteria Phylum: Firmicutes Class: Bacilli Order: Bacillales Family: Bacillaceae Genus: Bacillus Species Group: Bacillus cereus	1 x 3-4 µm, Gram-positive, rod-shaped,	structure of B. cereus consists of 5,717 genes and 5,265 coding proteins, Pseudo Genes: 326, rRNAs: 15 (5S, 16S, 23S),tRNAs: 104. The GC content of Bacillus cereus is about 35.4% . Note:Genes present within the chromosome associated with B. cereus virulence include genes encoding for non-hemolytic enterotoxins, channel-forming type III hemolysins, phospholipase C, a perfringolysin O (listeriolysin O), and extracellular proteases. All three proteins of the hemolysin BL enterotoxins associated with food poisoning.	isolated form different type source sucha as Spices,rice dish,reheated pasta..Pathological factor responsible for food born illness are toxic factor: Enterotoxin secrted during vegetative growth of B.cereus in small intestine while Emetic toxin secreted before ingestion within food source. These Pathalogical factor are leads to cause systemic and local infection such as : Diarrhoeal syndrome Emetic syndrome Eye infection Urinary Tract Infections Puerperal sepsis Pulmonar Disease.	damage stimulating degranulation human neutrop and breaks do the subepithe matrix affecti the healing tissue in infectio
Genus: Clostridium	bacteria ,rod-shaped,0.5-0.8 µm	Chromosomal neurotoxin gene clusters are found at one of three sites	Neurological disorders, Diarrhea disorders.	paralysis by inhibiting muscle contraction.
Species: Brucella melitensis	cocobacilli, or short rods		Insomnia-Depression,Arthritis.	intracellularly once inside a host.
Species: C. Jejuni.	curved and rod-shaped	is about 30.5% and the percentage coding of the bacteria is about 93%.		are likely to have a deleterious effect.
Genus:Enterobacteriaceae; Species Escherichia; E. coli	0.25–1.0 µm ,Rod-shaped,	bacterium in the stool which is essential for public health purposes, such as finding outbreaks	Cholecystitis, Colitis, Hemolytic Uremic Syndrome, Diarrheal Diseases.	information is availab for other EHEC serotypes.
Division: Firmicutes Class: Bacilli Order: Bacillales Family: Listeriaceae Genus: Listeria Species: L. monocytogenes	0.5 to 2 µm, rod-shaped	percentage coding of the bacteria is about 96.08%. Note: Surprisingly, many encoded proteins are similar to those of the soil bacterium Bacillus subtilis.	Miscarriage, Around 300 deaths are caused by Listeria infection each year, according to estimates from a 2011 CDC report.	bacterium a produces a Zn-dependent protease whi may act as sor sort of exotoxin.
Family: Enterobacteriaceae Genus: Salmonella	cylindrical rod of size	percentage coding of the bacteria is about 97.01%.	Septicaemia, Aortitis, Cholecystitis, Colitis	major contribution to pathogenesis.
Genus: Staphylococcus		aurenus is about 33% and the percentage	Respiratory Tract	using tampons during

Table 4.3: Extraction of Genome::ID along with Genome information

4.5.4.Preparation of Database of organisms with their genome.

An Excel file was created which contained all the information about the organism along with the information of their genome i.e., Genome ID and pathogenic: gene ID

Organism	Pathogenic factor	gene ID
Clostridium botulinum	Botulinum toxin	5185061
Listeria monocytogenes	Listeriolysin-O	14824605

Table 4.3 – An example of the dataset created for the organism with their genome and Pathogenic factor Gene ID.

4.5.5.STEP 5: Finding Oligo-Probe & primers.

4.5.5.1.Finding Oligo-Probe

.The pathogenic genes of all the organisms were assembled along with their genomes. Out of these pathogens, bacteria and Norovirus were selected for the dataset. Bacterial pathogenic gene were selected on basis of their toxicity in case of virus (Norovirus) whole genome sequence were used to find oligo- Probe by using universal probe library.

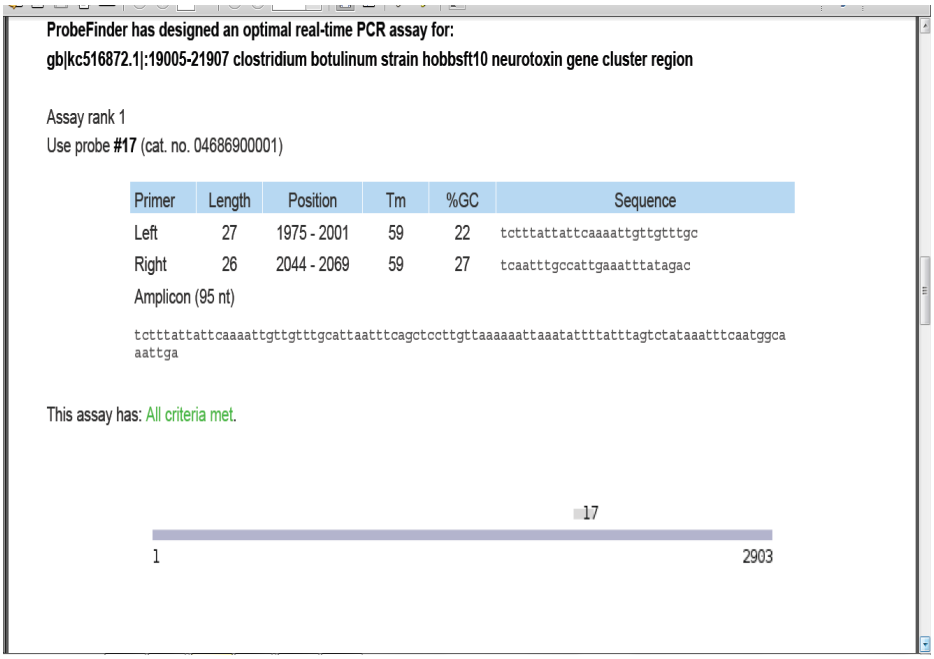

Figure 4.1 .The results generated by UPL.

Result generated by considering all the set parameters like Tm, CG% ,Primer length.

4.5.6. Finding species specific probes:

Universal Probe Library were compared in the NCBI Blast. In bioinformatics, Basic Local Alignment Search Tool.A BLAST search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold. Different types of BLAST are available according to the query sequences. The query sequences were entered in the BLAST. Now the BLAST search engine compared the query sequence with its database and the similar sequences were

found. The sequences which have 100 % identity with the bacteria selected were taken, If similarity less than 75% was found in the organism or high identity occurred in other organism, the sequences were discarded.

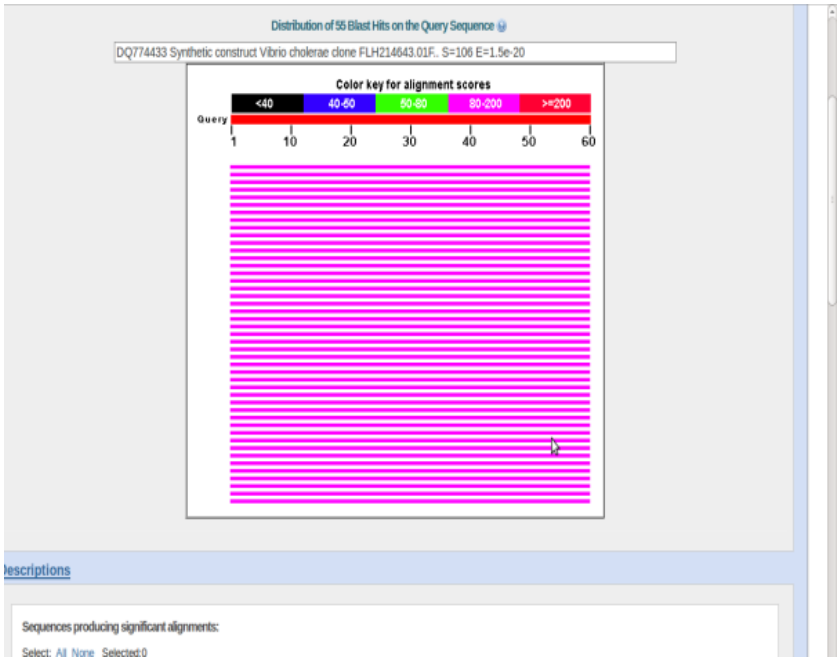

Figure 4.2.:BLAST run Result

BLAST Run to identify amplicon identify most of the sequences found had more than 90% identity in the DNA of the organisms taken.

4.5.7. Database of primers:

An Excel sheet was created for the primers. The columns included were organism's name, Amplicon, Left primer, Right primer, Probe, Temperature for both left and right primers, GC% for both left and right primers.

Organism	**Amplicon**	**Left Primer**	**Right Primer**	**Probe**	**Temperature(L/R)**	**GC% (L/R)**
Clostridium botulinum	tatggcggtgatggtaacgataagttgattgggggagcaggtaataattacctgaacggcggaga	Tctttattattcaaaattgttgtttgc	tcaatttgccattgaaatttatagac	cagctcct	59/59	22/27

.

Table 4.4: Validated *Clostridium botulinum* specific probe & primers (*in silico*

4.5.8 STEP 7: Preparation of database of species specific probe & primers

4.5.9. Web Implementation:

4.5.8.1 HTML

HTML was used for the development of our tool. HTML, stands for Hypertext Markup Language, is the predominant markup language for web pages. It simply describes contents of web pages and its structure. HTML cannot be used to write programs and it cannot control the precise layout of a web page. Web browsers are used to view HTML documents. It provides a means to create structured documents by denoting structural semantics for text such as headings, paragraphs, lists etc as well as for links, quotes, and other items. It allows images and objects to be embedded and can be used to create interactive forms. It is written in the form of HTML elements consisting of "tags" surrounded by angle brackets within the web page content. It can include or can load scripts in languages such as Java Script which affect the behavior of HTML processors like Web browser; and Cascading Style Sheet (CSS) to define the appearance and layout of text and other material. The W3C, maintainer of both HTML and CSS standards, encourages the use of CSS over explicit presentational markup.

4.5.8.2 CGI

CGI script is used to make the user access the information online. CGI stands for common gateway interface. CGI, a programming interface between a web server and the systems back end functions such as processing systems and databases. It defines how information is passed from the client's browser to the web server. The client script may directly reference the server

side script. CGI allows web servers to perform data functions and interact with users. CGI is required in order for information passed from the client to the server using HTTP to be processed by a program which is running on the server. The CGI defines a set of environment variables that are used to pass the data. The CGI variables may be set on the client side using client side embedded script code such as JAVA script. The Defined method for getting data from an HTML page which may include a script form is to use the GET or POST method.

The HTML file that is sent to and displayed on the user's web browser contains the server side script program reference. Therefore, following two items are required to get form data from the user to the server: An HTML FORM with the action attributes set to the location and name of the server side script. A client side script may also support the FORM by making sure entries are correct when the "Submit" button is clicked.

4.5.8.3 *Server side script*

When the CGI program produces output to its standard output stream, the web server program, such as Apache will send the data from the script program to the client's web browser. The requirement for this to happen is that the server side script program first output a Content-type header followed by a new line character:

An example of the header in Perl is:

Print "Content-type: text/html","\n";

When this header is output by the server side script program the web server will provide the rest of the required HTTP headers.

4.5.8.4 *PERL*

PERL stands for Practical Extraction and Report Language. By definition, PERL is a general purpose, interactive and dynamic programming language. Perl was originally developed by Larry Wall in the year 1987. Perl provides really strong data manipulation tools that make it one of the ideal languages to handle text files. Apart from handling text files, it is also used today more often for graphics programming. It is used for a variety of tasks that may include web development, network programming, Graphical User Interface development and system administration, applications that require database access and CGI programming on the Web. Perl is a very flexible language, allowing multiple ways of achieving the same outcome, which makes it very adaptive to your skill level. Per programming can also be used to read excel file. In our study, both the excel sheets created for the organisms with their genome ID and gene ID was extracted using perl programming.

Parse Excel function can be used to read the excel files. With the use of CGI script, these files were implemented on the web.

Figure 4.3.The use of the parse excel function

5. RESULTS

There are an enormous number of microbial pathogen. We have considered Food-borne pathogens are the known to cause food-borne illness (WHO guided lines; Appendix; 1). Mainly food-borne illness is associated with infection caused by microbial pathogens that have entered the food chain Microbe or toxin enters the body through the gastrointestinal tract, the most common symptoms of these illnesses are nausea, vomiting, diarrhoea and abdominal cramps. However, food-borne illness can vary dramatically in terms of how soon symptoms begin after eating or drinking the contaminated food, the length of illness .Pathogens have varying onset time fallowed by symptom's We Extracted information regarding their genome information ,size ,food source, pathogenesis and their associated disease and symptoms fallowed by them. (Mentioned in Appendix;2,3,4.).

A database is therefore created to get information of all food- pathogens and their nucleotide sequences. Small specific sequence portion for bacteria, also known as amplicons (primers & probes) were generated. These specific sequences are species specific and found only in relative bacterial species. These amplicons are extracted from pathogenic region of bacteria sequence act as specific regions and amplicons created from these portions result in specific detection of the species. This was done by using UPL primer designing tool.

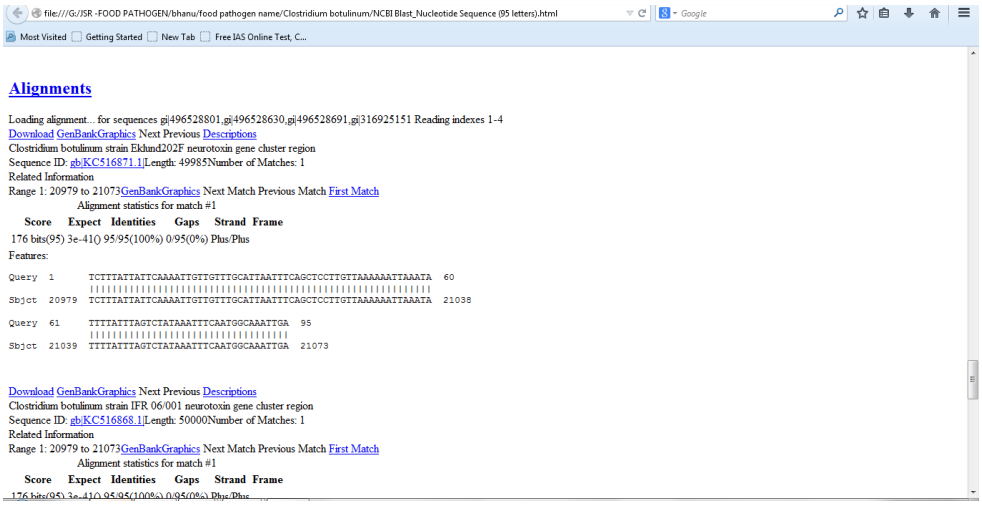

Figure 5.1 – Results generated by BLAST

BLAST run generated significant for the probe has been obtained has sequence identity These contained sequence information of both left and right primers, their melting temperature, GC content percentage and the most specific probe sequence for each amplicon

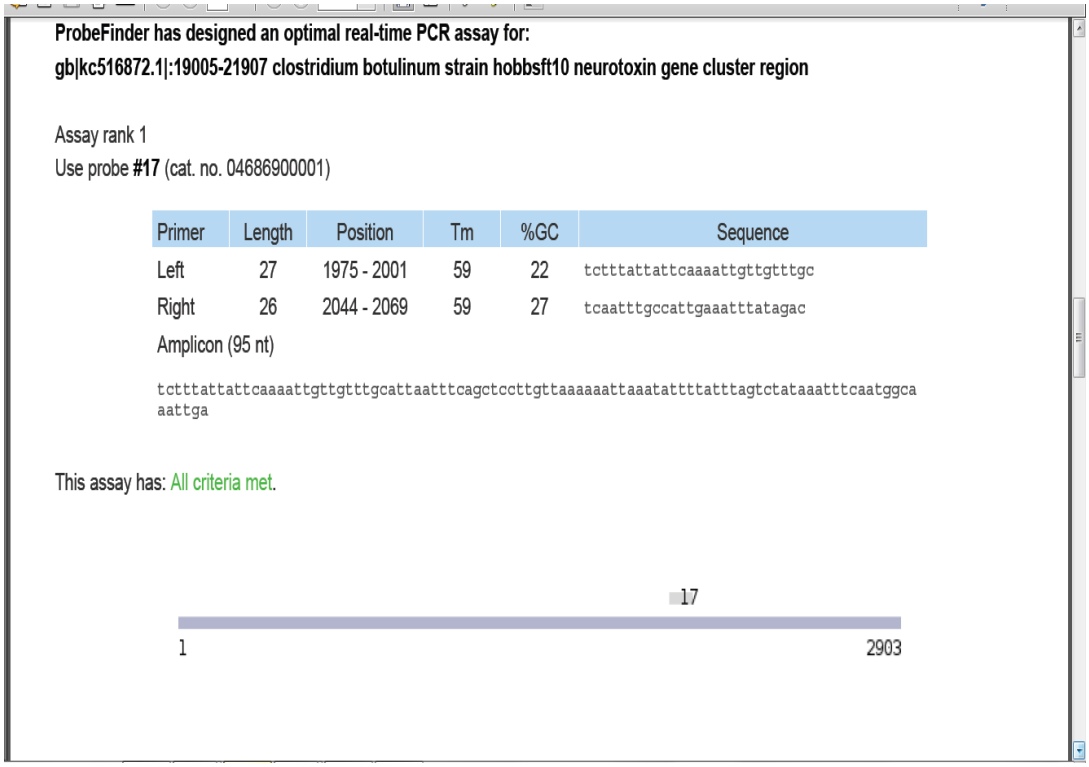

Figure 5.2 - Results generated by UPL for *campylobacter botulism*

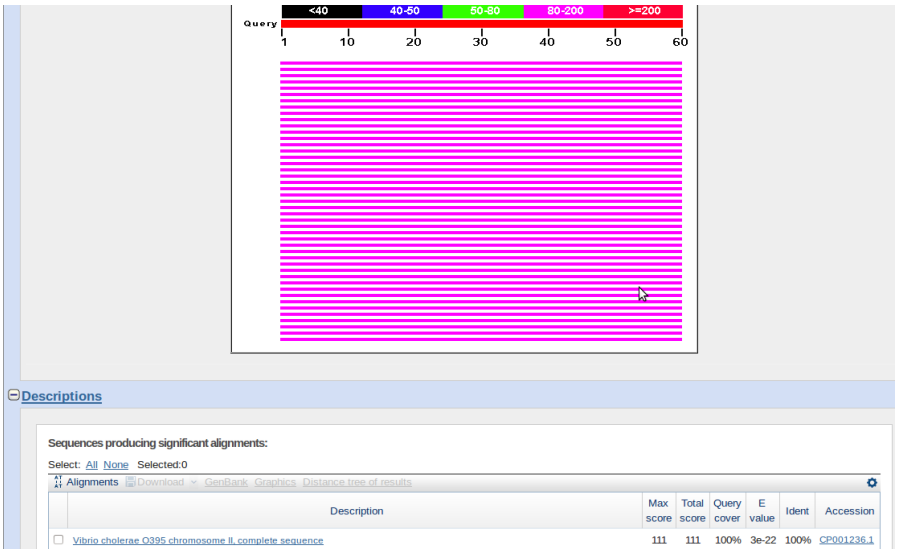

Figure 5.3. Results of BLAST of the amplicon generated by UPL for *campylobacter botulism.* Most of the sequences found had more than 90% identity in the DNA of the organisms taken.

Finally, a web-tool was developed named 'Food Pathogen'. It stands for Database of Primer and Probes to Detects Food Pathogens'. It is a user friendly tool and any one can access probe/primer from this web-tool as well as it has all the staple information for bacterial food pathogen .while in case of protozoan's and nematode and few fungi is updating is still going on web- tool. Bacterail pathogen Snapshot were taken as eg.

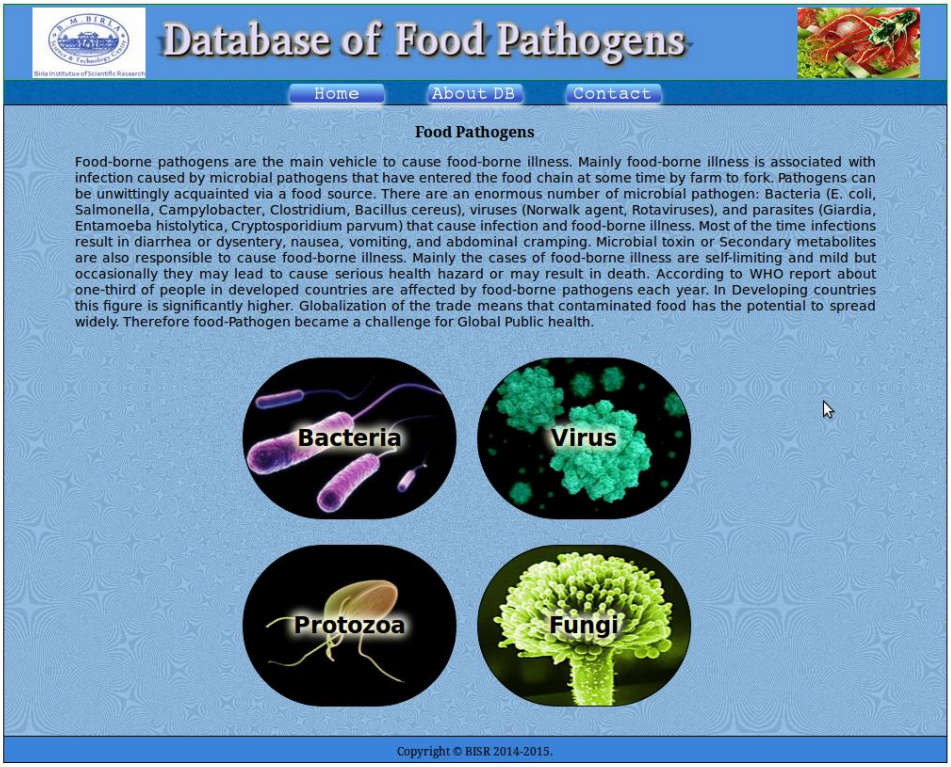

Figure 5.4:web tool creat

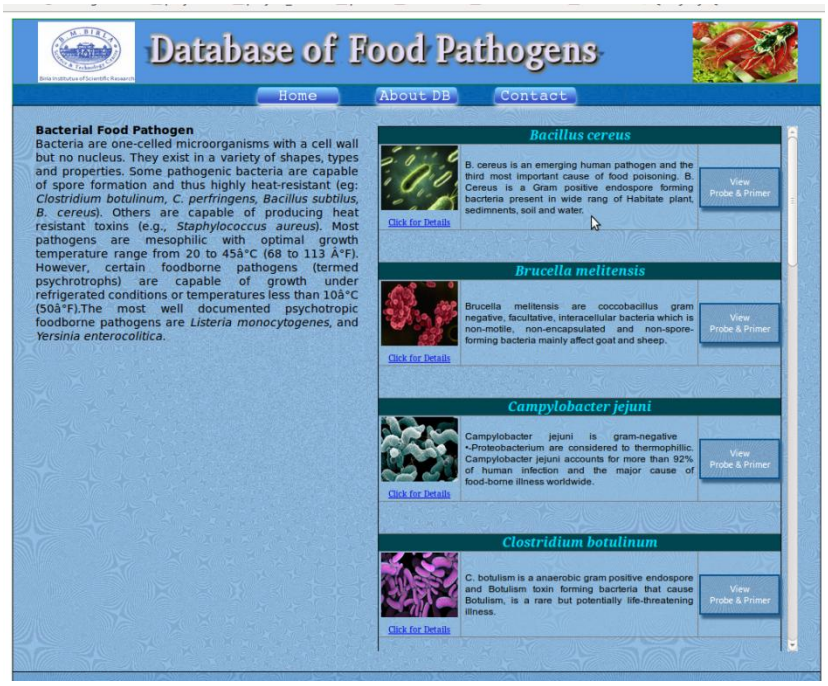

Figure 5.4. Web tool created for bacteria

The tool contains two columns. One has the list of brief account on species specific bacterial and also had the link to get detailed account on species specific pathogenic , and probe/primer

.pathogen while other side had generalized information about bacterial pathogen as shown below via taking bacillus as

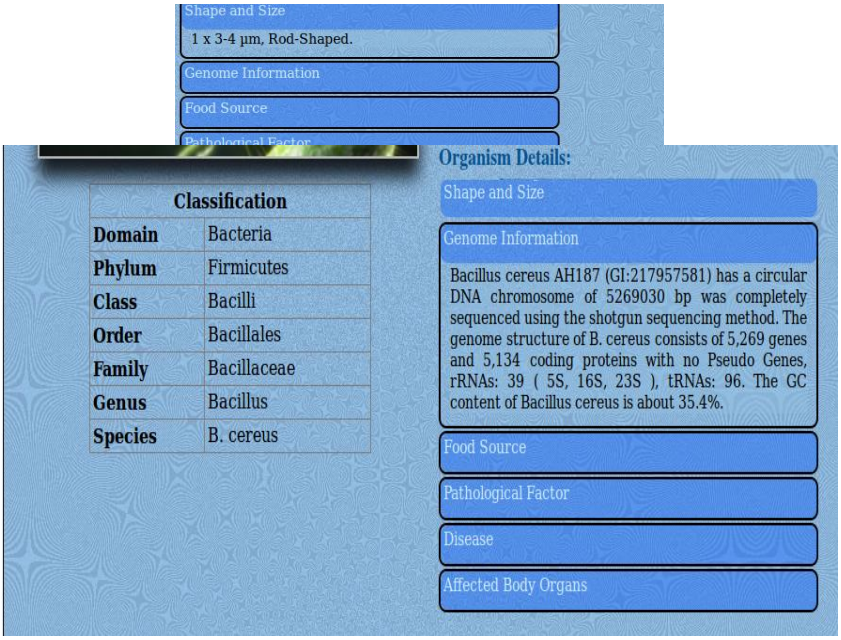

Figure 5.5 :Example Bacillus classification and Genome information and size.

Figure 5.6. Generated Primers and probes

7. CONCLUSION AND DISCUSSION

Food-borne pathogens are the main vehicle to cause food-borne illness. Mainly food-borne illness is associated with infection caused by microbial pathogens that have entered the food chain at some time by farm to fork. In 2014 it was estimated that foodborne pathogens were responsible for 76 million illnesses annually, resulting in 5,000 deaths. This report identified Salmonella, Listeria and Toxoplasma as the major causative agents, being responsible for 1,500 of the reported deaths. Data published in 2006 by the CDC suggested that infections due to Yersinia, Shigella, Listeria, Campylobacter, Escherichia coli O157:H7 and Salmonella have decreased dramatically, while infections due to Vibrio have also creased . and E. coli O157:H7 are likely a result of increased awareness. The FDA, USDA and EU have all implemented a zero-tolerance rule for L. monocytogenes in ready-to-eat. (CDC;Fact sheet.2014).

Some pathogenic bacteria are capable of spore formation and thus highly heat-resistant *(e.g., Clostridium botulinum, C. perfringens, Bacillus subtilus, B. cereus*). Others are capable of producing heat-resistant toxins (e.g., *Staphylococcus aureus*). Most pathogens are mesophilic with optimal growth temperature range from 20 to 45^0C (68 to 113^0F). However, certain foodborne pathogens (termed psychrotrophs) are capable of growth under refrigerated conditions or temperatures less than 10^0C (50^0F). The most well documented psychotropic foodborne pathogens are Listeria monocytogenes, and Yersinia enterocolitica. Listeria monocytogenes, for example, will grow (albeit slowly) at temperatures just of above freezing (approximately 33-34^0F). Certain strains or serotypes of Bacillus cereus, Clostridium botulinum, Salmonella spp., E. coli O157:H7, and Staphylococcus aureus may also grow slowly under refrigeration conditions. Bacteria also vary in (Ronald *et.al* 2012). Protozoans are found as opportunistic food pathogen. Fungi metabolites known for their potent carcinogenic properties. The ability of aflatoxin production has been reported in various species of the *Aspergillus* genus. .(Mohamed E. Zain;2011).

Bacterial pathogens damage the host via invasive and toxic attributes.(James et.al 2010). Fungi metabolic process, moulds produce mycotoxins. Those natural products, poisonous to humans and animals, are created as the result of a secondary metabolic process of fungi(Mohamed E. Zain.2011).Protozoan's are opportunistic food pathogen that causes severe disease in immunocompromised patients as *T. gondii* widespread three lineages when grown on organic substrates. Norovirus is emerging pathogenic calicivirus, recognized as the leading cause of gastroenteritis and of food-related outbreaks.

Bioinformatics allows us to predict the specific primers and probes for the organisms with a good accuracy and high speed i.e., *insilico* prediction. In the current project, various bioinformatics tools have been used to create a dataset of primers and probes from the variable Pathogenic regions of the organisms which can be used for the specific regions detection of the bacteria responsible to ca use foodborne. Using this dataset a user friendly web tool has been created. By using this tool a user can get information of water pathogens

and the specific nucleotide sequences that they have. A research scholar can take advantage of this tool as it contains unique sequences of primers and probes for all bacteria and can use these sequences in PCR for Pathogen detection in to detection .

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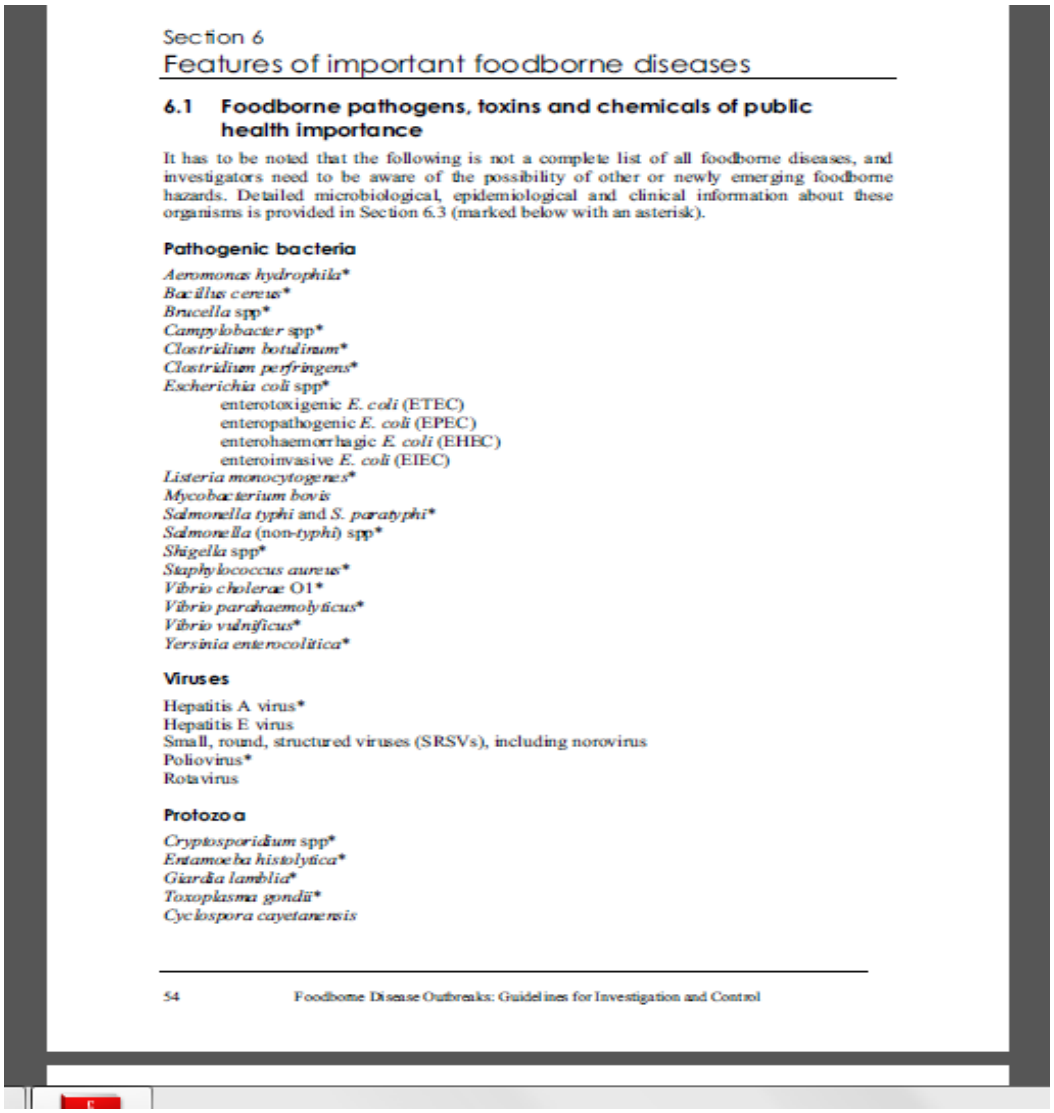
APPENDIX



Section 6

Features of important foodborne diseases

6.1 Foodborne pathogens, toxins and chemicals of public health importance

It has to be noted that the following is not a complete list of all foodborne diseases, and investigators need to be aware of the possibility of other or newly emerging foodborne hazards. Detailed microbiological, epidemiological and clinical information about these organisms is provided in Section 6.3 (marked below with an asterisk).

Pathogenic bacteria

*Aeromonas hydrophila**
*Bacillus cereus**
Brucella spp*
Campylobacter spp*
*Clostridium botulinum**
*Clostridium perfringens**
Escherichia coli spp*
- enterotoxigenic *E. coli* (ETEC)
- enteropathogenic *E. coli* (EPEC)
- enterohaemorrhagic *E. coli* (EHEC)
- enteroinvasive *E. coli* (EIEC)

*Listeria monocytogenes**
Mycobacterium bovis
Salmonella typhi and *S. paratyphi**
Salmonella (non-*typhi*) spp*
Shigella spp*
*Staphylococcus aureus**
Vibrio cholerae O1*
*Vibrio parahaemolyticus**
*Vibrio vulnificus**
*Yersinia enterocolitica**

Viruses

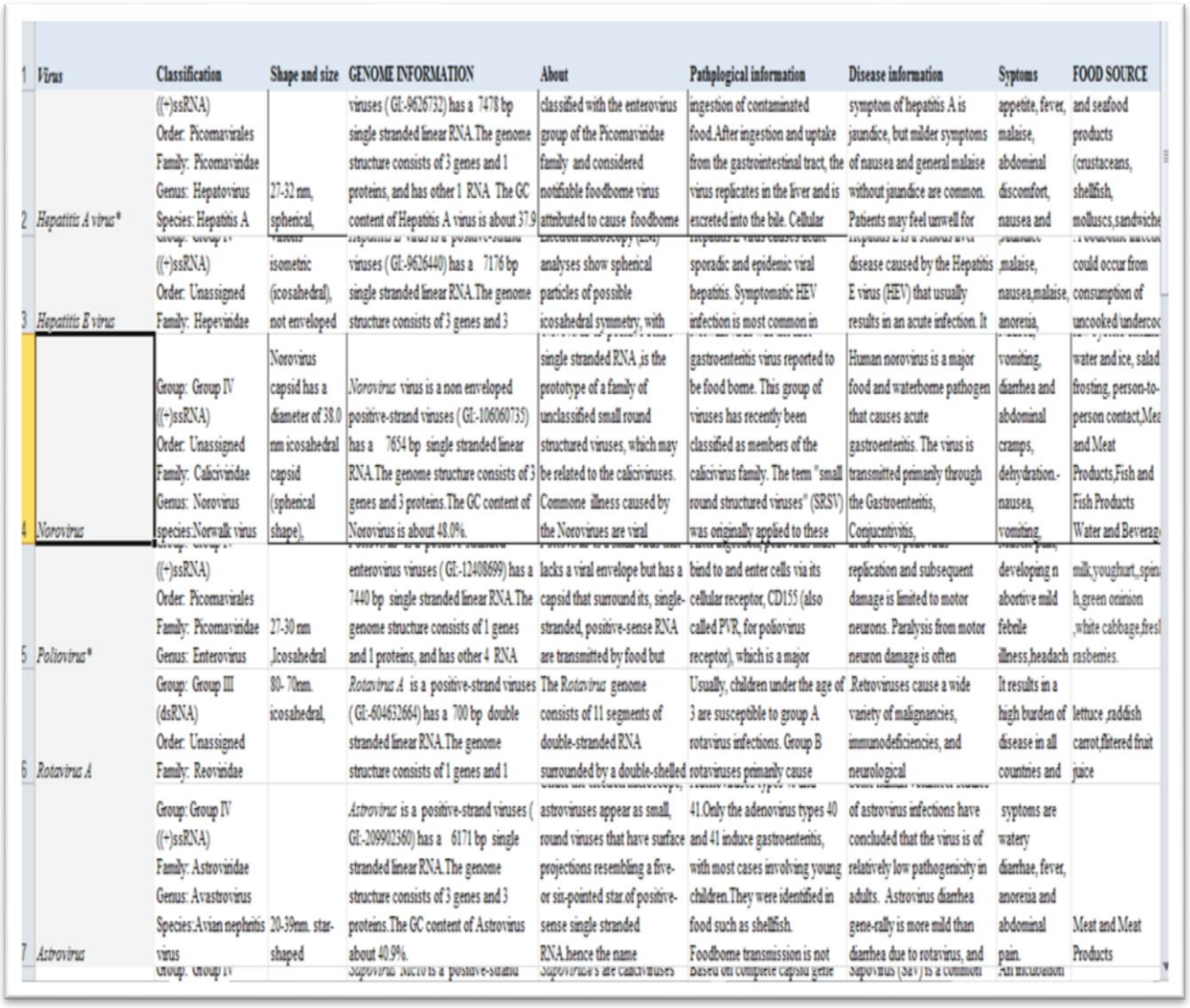
Hepatitis A virus*
Hepatitis E virus
Small, round, structured viruses (SRSVs), including norovirus
Poliovirus*
Rotavirus

Protozoa

Cryptosporidium spp*
*Entamoeba histolytica**
*Giardia lamblia**
*Toxoplasma gondii**
Cyclospora cayetanensis

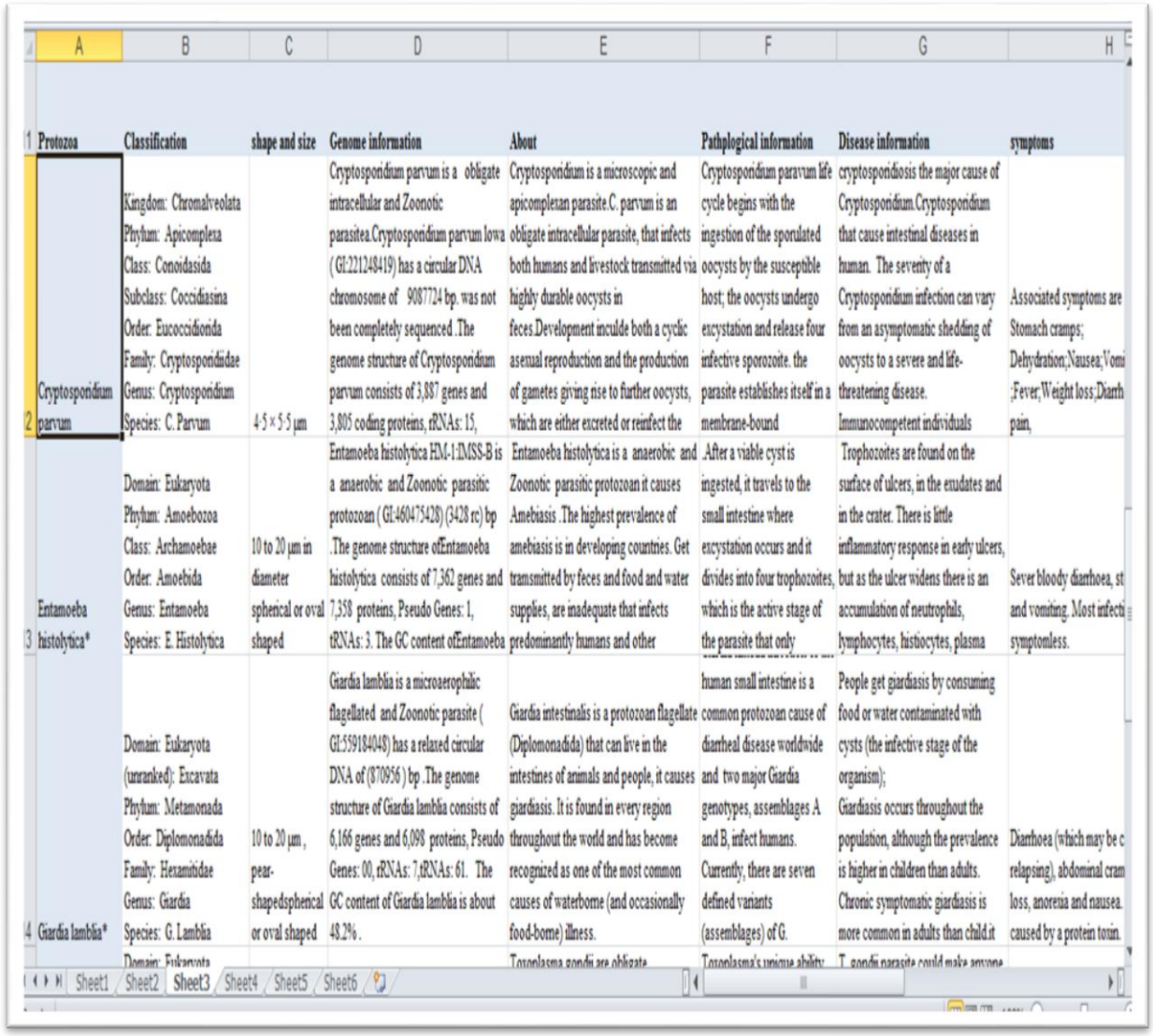
54 Foodborne Disease Outbreaks: Guidelines for Investigation and Control

Appendix.1



	Virus	Classification	Shape and size	GENOME INFORMATION	About	Pathological information	Disease information	Symtoms	FOOD SOURCE
2	*Hepatitis A virus**	((+)ssRNA) Order: Picornavirales Family: Picornaviridae Genus: Hepatovirus Species: Hepatitis A	27-32 nm, spherical,	viruses (GI:-9626732) has a 7478 bp single stranded linear RNA.The genome structure consists of 3 genes and 1 proteins, and has other 1 RNA The GC content of Hepatitis A virus is about 37.9	classified with the enterovirus group of the Picornaviridae family and considered notifiable foodborne virus attributed to cause foodborne	ingestion of contaminated food.After ingestion and uptake from the gastrointestinal tract, the virus replicates in the liver and is excreted into the bile. Cellular	symptom of hepatitis A is jaundice, but milder symptoms of nausea and general malaise without jaundice are common. Patients may feel unwell for	appetite, fever, malaise, abdominal discomfort, nausea and	and seafood products (crustaceans, shellfish, molluscs,sandwiche
3	*Hepatitis E virus*	((+)ssRNA) Order: Unassigned Family: Hepeviridae	isometric (icosahedral), not enveloped	viruses (GI:-9626440) has a 7176 bp single stranded linear RNA.The genome structure consists of 3 genes and 3	analyses show spherical particles of possible icosahedral symmetry, with	sporadic and epidemic viral hepatitis. Symptomatic HEV infection is most common in	disease caused by the Hepatitis E virus (HEV) that usually results in an acute infection. It	,malaise, nausea,malaise, anorexia,	could occur from consumption of uncooked/undercoo
4	*Norovirus*	Group: Group IV ((+)ssRNA) Order: Unassigned Family: Caliciviridae Genus: Norovirus species:Norwalk virus	Norovirus capsid has a diameter of 38.0 nm icosahedral capsid (spherical shape),	*Norovirus* virus is a non enveloped positive-strand viruses (GI:-106060735) has a 7654 bp single stranded linear RNA.The genome structure consists of 3 genes and 3 proteins.The GC content of Norovirus is about 48.0%.	single stranded RNA ,is the prototype of a family of unclassified small round structured viruses, which may be related to the caliciviruses. Commone illness caused by the Norovirues are viral	gastroenteritis virus reported to be food borne. This group of viruses has recently been classified as members of the calicivirus family. The term "small round structured viruses" (SRSV) was originally applied to these	Human norovirus is a major food and waterborne pathogen that causes acute gastroenteritis. The virus is transmitted primarily through the Gastroenteritis, Conjunctivitis,	vomiting, diarrhea and abdominal cramps, dehydration.- nausea, vomiting,	water and ice, salad frosting, person-to-person contact,Mea and Meat Products,Fish and Fish Products Water and Beverag
5	*Poliovirus**	((+)ssRNA) Order: Picornavirales Family: Picornaviridae Genus: Enterovirus	27-30 nm ,Icosahedral	enterovirus viruses (GI:-12408699) has a 7440 bp single stranded linear RNA.The genome structure consists of 1 genes and 1 proteins, and has other 4 RNA	lacks a viral envelope but has a capsid that surround its, single-stranded, positive-sense RNA are transmitted by food but	bind to and enter cells via its cellular receptor, CD155 (also called PVR, for poliovirus receptor), which is a major	replication and subsequent damage is limited to motor neurons. Paralysis from motor neuron damage is often	developing n abortive mild febrile illness,headach	milk,youghurt,,spin h,green onion ,white cabbage,fres rasberies.
6	*Rotavirus A*	Group: Group III (dsRNA) Order: Unassigned Family: Reoviridae	80- 70nm. icosahedral,	*Rotavirus A* is a positive-strand viruses (GI:-604632664) has a 700 bp double stranded linear RNA.The genome structure consists of 1 genes and 1	The *Rotavirus* genome consists of 11 segments of double-stranded RNA surrounded by a double-shelled	Usually, children under the age of 3 are susceptible to group A rotavirus infections. Group B rotaviruses primarily cause	.Retroviruses cause a wide variety of malignancies, immunodeficiencies, and neurological	It results in a high burden of disease in all countries and	lettuce ,raddish carrot,filtered fruit juice
7	*Astrovirus*	Group: Group IV ((+)ssRNA) Family: Astroviridae Genus: Avastrovirus Species:Avian nephritis virus	20-39nm. star-shaped	*Astrovirus* is a positive-strand viruses (GI:-209902360) has a 6171 bp single stranded linear RNA.The genome structure consists of 3 genes and 3 proteins.The GC content of Astrovirus about 40.9%.	astroviruses appear as small, round viruses that have surface projections resembling a five- or six-pointed star.of positive-sense single stranded RNA,hence the name	41.Only the adenovirus types 40 and 41 induce gastroenteritis, with most cases involving young children.They were identified in food such as shellfish. Foodborne transmission is not	of astrovirus infections have concluded that the virus is of relatively low pathogenicity in adults. Astrovirus diarrhea gene-rally is more mild than diarrhea due to rotavirus, and	syptoms are watery diarrhae, fever, anorexia and abdominal pain.	Meat and Meat Products

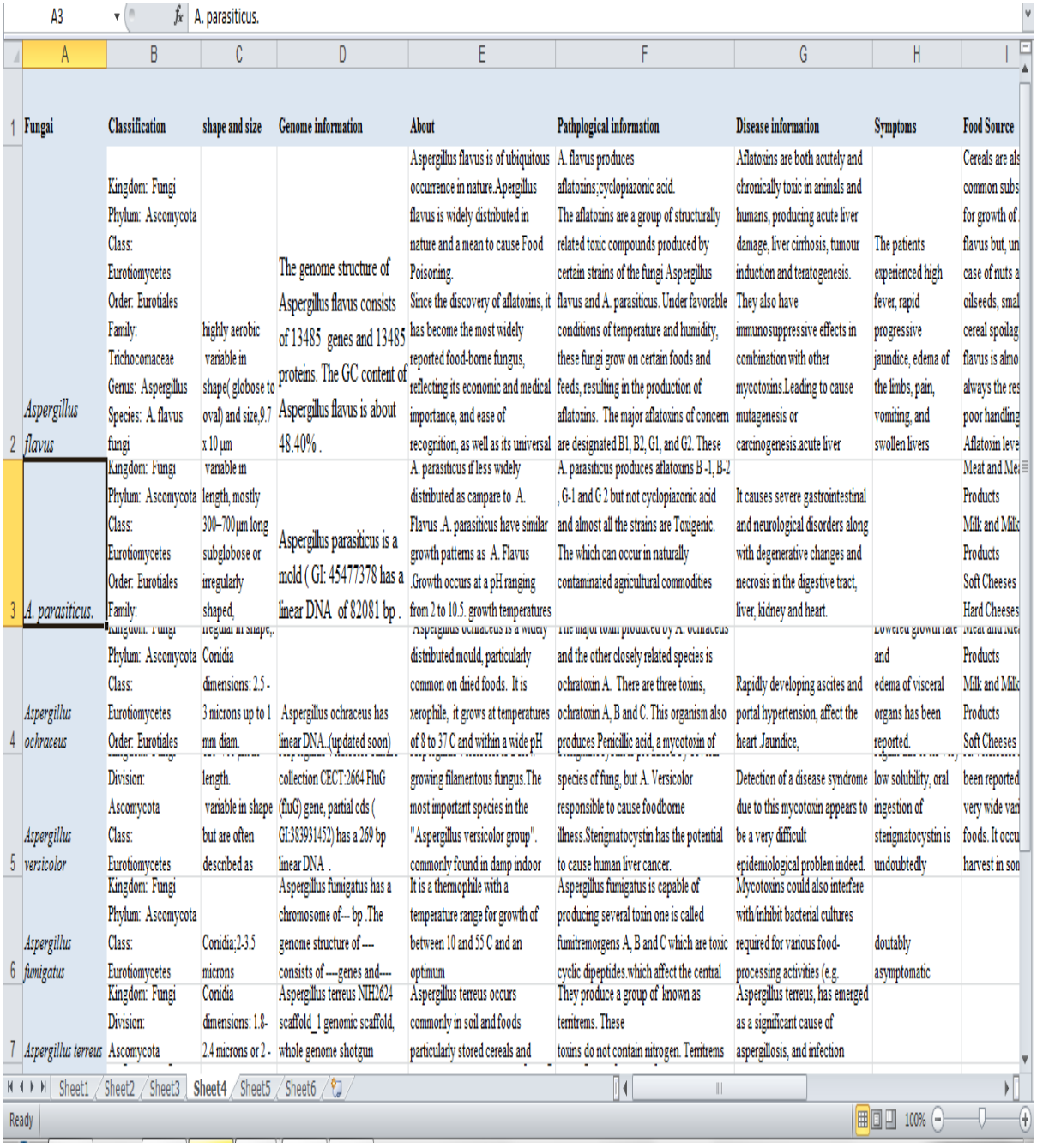
Appendix.2



	A	B	C	D	E	F	G	H
1	Protozoa	Classification	shape and size	Genome information	About	Pathological information	Disease information	symptoms
2	Cryptosporidium parvum	Kingdom: Chromalveolata Phylum: Apicomplexa Class: Conoidasida Subclass: Coccidiasina Order: Eucoccidiorida Family: Cryptosporidiidae Genus: Cryptosporidium Species: C. Parvum	4·5 × 5·5 µm	Cryptosporidium parvum is a obligate intracellular and Zoonotic parasitea.Cryptosporidium parvum Iowa (GI:221248419) has a circular DNA chromosome of 9087724 bp. was not been completely sequenced .The genome structure of Cryptosporidium parvum consists of 3,887 genes and 3,805 coding proteins, rRNAs: 15,	Cryptosporidium is a microscopic and apicomplexan parasite.C. parvum is an obligate intracellular parasite, that infects both humans and livestock transmitted via highly durable oocysts in feces.Development inculde both a cyclic asexual reproduction and the production of gametes giving rise to further oocysts, which are either excreted or reinfect the	Cryptosporidium paravum life cycle begins with the ingestion of the sporulated oocysts by the susceptible host; the oocysts undergo excystation and release four infective sporozoite. the parasite establishes itself in a membrane-bound	cryptosporidiosis the major cause of Cryptosporidium.Cryptosporidium that cause intestinal diseases in human. The seveirty of a Cryptosporidium infection can vary from an asymptomatic shedding of oocysts to a severe and life-threatening disease. Immunocompetent individuals	Associated symptoms are Stomach cramps; Dehydration;Nausea;Vomi ;Fever,Weight loss;Diarrh pain,
3	Entamoeba histolytica*	Domain: Eukaryota Phylum: Amoebozoa Class: Archamoebae Order: Amoebida Genus: Entamoeba Species: E. Histolytica	10 to 20 µm in diameter spherical or oval shaped	Entamoeba histolytica HM-1:IMSS-B is a anaerobic and Zoonotic parasitic protozoan (GI:460475428) (3428 rc) bp .The genome structure ofEntamoeba histolytica consists of 7,362 genes and 7,358 proteins, Pseudo Genes: 1, tRNAs: 3. The GC content ofEntamoeba	Entamoeba histolytica is a anaerobic and Zoonotic parasitic protozoan it causes Amebiasis .The highest prevalence of amebiasis is in developing countries. Get transmitted by feces and food and water supplies, are inadequate that infects predominantly humans and other	.After a viable cyst is ingested, it travels to the small intestine where excystation occurs and it divides into four trophozoites, which is the active stage of the parasite that only	Trophozoites are found on the surface of ulcers, in the exudates and in the crater. There is little inflammatory response in early ulcers, but as the ulcer widens there is an accumulation of neutrophils, lymphocytes, histiocytes, plasma	Sever bloody diarrhoea, st and vomiting. Most infecti symptomless.
4	Giardia lamblia*	Domain: Eukaryota (unranked): Excavata Phylum: Metamonada Order: Diplomonadida Family: Hexamitidae Genus: Giardia Species: G. Lamblia	10 to 20 µm , pear-shapedspherical or oval shaped	Giardia lamblia is a microaerophilic flagellated and Zoonotic parasite (GI:559184048) has a relazed circular DNA of (870956) bp .The genome structure of Giardia lamblia consists of 6,166 genes and 6,098 proteins, Pseudo Genes: 00, rRNAs: 7,tRNAs: 61. The GC content of Giardia lamblia is about 48.2% .	Giardia intestinalis is a protozoan flagellate (Diplomonadida) that can live in the intestines of animals and people, it causes giardiasis. It is found in every region throughout the world and has become recognized as one of the most common causes of waterborne (and occasionally food-borne) illness.	human small intestine is a common protozoan cause of diarrheal disease worldwide and two major Giardia genotypes, assemblages A and B, infect humans. Currently, there are seven defined variants (assemblages) of G.	People get giardiasis by consuming food or water contaminated with cysts (the infective stage of the organism); Giardiasis occurs throughout the population, although the prevalence is higher in children than adults. Chronic symptomatic giardiasis is more common in adults than child.it	Diarrhoea (which may be c relapsing), abdominal cram loss, anorexia and nausea. caused by a protein toxin.
5		Domain: Eukaryota			Toxoplasma gondii are obligate	Toxoplasma's unique ability	T. gondii parasite could make anyone	

Sheet1 Sheet2 Sheet3 Sheet4 Sheet5 Sheet6

Appendix.3



	Fungai	Classification	shape and size	Genome information	About	Pathlogical information	Disease information	Symptoms	Food Source
2	*Aspergillus flavus*	Kingdom: Fungi Phylum: Ascomycota Class: Eurotiomycetes Order: Eurotiales Family: Trichocomaceae Genus: Aspergillus Species: A. flavus fungi	highly aerobic variable in shape(globose to oval) and size,9.7 x 10 µm	The genome structure of Aspergillus flavus consists of 13485 genes and 13485 proteins. The GC content of Aspergillus flavus is about 48.40% .	Aspergillus flavus is of ubiquitous occurrence in nature.Apergillus flavus is widely distributed in nature and a mean to cause Food Poisoning. Since the discovery of aflatoxins, it has become the most widely reported food-borne fungus, reflecting its economic and medical importance, and ease of recognition, as well as its universal	A. flavus produces aflatoxins;cyclopiazonic acid. The aflatoxins are a group of structurally related toxic compounds produced by certain strains of the fungi Aspergillus flavus and A. parasiticus. Under favorable conditions of temperature and humidity, these fungi grow on certain foods and feeds, resulting in the production of aflatoxins. The major aflatoxins of concern are designated B1, B2, G1, and G2. These	Aflatoxins are both acutely and chronically toxic in animals and humans, producing acute liver damage, liver cirrhosis, tumour induction and teratogenesis. They also have immunosuppressive effects in combination with other mycotoxins.Leading to cause mutagenesis or carcinogenesis.acute liver	The patients experienced high fever, rapid progressive jaundice, edema of the limbs, pain, vomiting, and swollen livers	Cereals are als common subs for growth of flavus but, un case of nuts a oilseeds, smal cereal spoilag flavus is almo always the re poor handling Aflatoxin leve
3	*A. parasiticus.*	Kingdom: Fungi Phylum: Ascomycota Class: Eurotiomycetes Order: Eurotiales Family:	variable in length, mostly 300–700 µm long subglobose or irregularly shaped,	Aspergillus parasiticus is a mold (GI: 45477378 has a linear DNA of 82081 bp .	A. parasiticus tf less widely distributed as campare to A. Flavus .A. parasiticus have similar growth patterns as A. Flavus .Growth occurs at a pH ranging from 2 to 10.5. growth temperatures	A. parasiticus produces aflatoxins B -1, B-2 , G-1 and G 2 but not cyclopiazonic acid and almost all the strains are Toxigenic. The which can occur in naturally contaminated agricultural commodities	It causes severe gastrointestinal and neurological disorders along with degenerative changes and necrosis in the digestive tract, liver, kidney and heart.		Meat and Me Products Milk and Milk Products Soft Cheeses Hard Cheeses
4	*Aspergillus ochraceus*	Kingdom: Fungi Phylum: Ascomycota Class: Eurotiomycetes Order: Eurotiales	Irregular in shape,. Conidia dimensions: 2.5 - 3 microns up to 1 mm diam.	Aspergillus ochraceus has linear DNA..(updated soon)	Aspergillus ochraceus is a widely distributed mould, particularly common on dried foods. It is xerophile, it grows at temperatures of 8 to 37 C and within a wide pH	The major toxin produced by A. ochraceus and the other closely related species is ochratoxin A. There are three toxins, ochratoxin A, B and C. This organism also produces Penicillic acid, a mycotoxin of	Rapidly developing ascites and portal hypertension, affect the heart .Jaundice,	Lowered growth rate and edema of visceral organs has been reported.	Meat and Me Products Milk and Milk Products Soft Cheeses
5	*Aspergillus versicolor*	Division: Ascomycota Class: Eurotiomycetes	length. variable in shape but are often described as	collection CECT:2664 FluG (fluG) gene, partial cds (GI:383931452) has a 269 bp linear DNA .	growing filamentous fungus.The most important species in the "Aspergillus versicolor group". commonly found in damp indoor	species of fung, but A. Versicolor responsible to cause foodborne illness.Sterigmatocystin has the potential to cause human liver cancer.	Detection of a disease syndrome due to this mycotoxin appears to be a very difficult epidemiological problem indeed.	low solubility, oral ingestion of sterigmatocystin is undoubtedly	been reported very wide var foods. It occu harvest in son
6	*Aspergillus fumigatus*	Kingdom: Fungi Phylum: Ascomycota Class: Eurotiomycetes	Conidia;2-3.5 microns	Aspergillus fumigatus has a chromosome of--- bp .The genome structure of ---- consists of ----genes and----	It is a thermophile with a temperature range for growth of between 10 and 55 C and an optimum	Aspergillus fumigatus is capable of producing several toxin one is called fumitremorgens A, B and C which are toxic cyclic dipeptides.which affect the central	Mycotoxins could also interfere with/inhibit bacterial cultures required for various food-processing activities (e.g.	doutably asymptomatic	
7	*Aspergillus terreus*	Kingdom: Fungi Division: Ascomycota	Conidia dimensions: 1.8-2.4 microns or 2 -	Aspergillus terreus NIH2624 scaffold_1 genomic scaffold, whole genome shotgun	Aspergillus terreus occurs commonly in soil and foods particularly stored cereals and	They produce a group of known as territrems. These toxins do not contain nitrogen. Territrems	Aspergillus terreus, has emerged as a significant cause of aspergillosis, and infection		

Appendix.4.