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Review on Molecular Detection of *Salmonella typhimurium*

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Abstract: *Salmonella typhimurium*is one of the non-host-adapted serovars that cause food poisoning in humans, gastroenteritis in both humans and other mammals. This serovars is Gram negative predominantly found in the intestinal lumen. Its toxicity is due to an outer membrane consisting largely lipopolysaccharides (LPS). The epidemiology of *Salmonella typhimurium* is worldwide. But, the epidemiological patterns of prevalence and incidence of disease differ greatly between geographical areas depending up on climate, food harvesting and processing technology, and consumer habits. The disease is primarily caused by the consumption of contaminated food and water but may also be transmitted by fecal–oral route and direct contact with infected animals. Diversity among S*almonella* has been studied using various phenotypic methods including antimicrobial resistance testing and phage typing, and by genotypic methods, including Pulsed Field Gel Electrophoresis (PFGE), Amplified Fragment Length Polymorphism (AFLP) and Repetitive extra genic palindromic polymerase chain reaction (Rep-PCR). A distinct strains of *Salmonella enterica serotypetyphimurium,* known as definitivetype104 (DT104) is resistant to certain antibiotics. Specific measures of *Salmonella typhimurium* prevention and control include thorough cooking of foods of animal origin, avoiding cross contamination with other foods, avoiding consumption of unpasteurized dairy products and educating food handlers.

Key words: *Salmonella typhimurium · Phenotypic Method · Genotypic Method · Drug resistance · Public health importance*

INTRODUCTION

The genus *Salmonella* is a member of the Family *Enterobacteriaceae*, and is composed of two species, *S. bongori* and *S. enterica* species that has been divided in to over 2000 serotypes in the Kauffman- White scheme, based on the O (Somatic), H (Flagellar), and occasionally capsular K (Vi) antigens. Of the serovars of this genus that had been identified up to now, only 20 belonged to *S. bongori* and the remaining is to *S. enterica* [1]. More recently *S. enterica* has been divided in to six subgroups. These are subgroup I (*S. entericasubspecies enterica*), subgroup II (*S. entericasubspecies salmonae*), subgroup IIIa (*S. entericasubspecies arizonae*), subgroup IIIb (*S. entericasubspecies diarizonae*), subgroup IV (*S. entericasubspecies houtenae*) and subgroup VI (*S. entericasubspecies indica*). Subgroup I contains most of Salmonellae that are significant to animal pathogens (e.g. *Typhimurium*) [2].

Diversity among salmonellae has been studied using various phenotypic methods, including antimicrobial resistance profiling and phage typing, and by genotypic methods including Pulsed Field Gel Electrophoresis (PFGE), Amplified Fragment Length Polymorphism (AFLP) and Repetitive extra genic palindromic polymerase chain reaction (Rep- PCR) [3]. Even though many serovars of *Salmonella enterica* have thus far been recognized [3], few serovars appear to cause most food - borne illness. One Non host - adapted serovars common to Food animals and humans and with wide distribution is *Typhimurium* [3].

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Salmonella typhimurium is a pathogenic Gram negative organism predominantly found in the intestinal lumen, its toxicity is due to an outer membrane consisting largely of lipopolysaccharides (LPS) which protect the bacteria form the environment. The LPS is made up of an O-antigen, a polysaccharide core, and lipid A which connects in to the outer membrane. Lipid A is made up of two phosphorylated glucosamine which are attached to fatty acids. Animals carry an enzyme that specifically removes these phosphate groups in an attempt to protect themselves from these pathogens. The O- antigen, being on the outermost parts of LPS complex is responsible for the host immune response. *S. typhimurium* has the ability to undergo acetylation of this antigen, which changes its conformation, and makes it difficult for antibodies to recognize [4, 5].

Movement is accomplished with aperitrichous flagella arrangement, but it does not produce endo-spores. Some of the metabolic features that can help to identify the bacteria include being lactose negative, citrate positive, lysine decarboxylase positive, gelatin hydrolysis negative, and the production hydrogen sulfide [6].

Strains of *Salmonella* that are resistant to antimicrobial agents have been a worldwide health problem. A distinct strain of *Salmonella enterica serotype Typhimurium,* known as definitive type 104 (D104) is resistant to certain antimicrobial agents [7].

Unique control methods are presently not available for S*almonella Typhimuriun* DT104 because little is known about the epidemiology of DT104. Food poisoning caused by this species of the genes *Salmonella* is prevented by attenuated *salmonella* vaccine strains which have been developed by introducing mutation in to the bacterium that diminish its overall growth [8].

Therefore, the objectives of this seminar paper were:

- To review the genotypic and phenotypic methods to identify *Salmonella typhimurium.*
- To discuss the source of infection and mode of transmission of *Salmonella Typhimurium.*
- To review the multidrug resistance pattern of *Salmonella typhimurium.*
- To describe the pathogenic effect of *Salmonella typhimurium* on cattle, sheep, goat, horse, swine and its public health importance.

EPIDEMIOLOGY OF SALMONELLA TYPHIMUNIUM

Incidence and Distribution: The epidemiology of *salmonella typhimurium* is worldwide but the epidemiological patterns of prevalence and incidence of Disease differ greatly between geographical areas depending up on climate, Food harvestings and processing technologies, and consumer habits. The incidence of non-typhoid salmonellosis (which is caused by *Salmonella typhimurium*) is increasing worldwide, causing millions of infections and many deaths in the animal population each year [9].

Source of Infection and Transmission: Salmonellae are carried in the intestinal tracts and associated organs of most farm and wild animals. They are spread by direct or indirect means. Infected animals are the source of the organisms which they excrete and infect other animals directly or indirectly by contamination of the environment, primarily feed and water supplies. Ingestion of infected feed and water or contact with contaminated execration is the main ways of infection [10].

Although salmonellae may survive for a long periods in the environment, it is the carrier state that provides the major source of infection for animals and human. Thus, persistence of infection in animals and in the environment is an important epidemiological feature of salmonellosis [10, 11].

The mode of *salmonella* transmission is complicated and a great number of animals, birds, and reptiles are responsible for the maintenance of the chain of infection. A farm animal may be infected from various sources, including food stuffs, birds, bedding, flies, rodents, sewage, soil, and water. During transportation to slaughter, there may be cross contamination of animals. There are opportunities for contamination at many stages during the slaughter dressing, and preparation of raw meats. These are very important sources of infection in human food chain [1, 12].

Transmission of *Salmonella typhimurium* sheep and goats is most commonly by the fecal oral route. The organism spreads geographically by the migration of carrier animal and by the transportation of contaminated feed and water. The introduction of unrecognized carrier animal in to susceptible population of sheep and goat is of major importance in the propagation and outbreaks of salmonellosis [13].

Host Range: *Salmonella typhimurium* can affect a wide host range of animals among which feral birds and rodents play important roles in interspecific dissemination of infection. Young debilitated and parturient animals are most susceptible to clinical disease. Man is highly susceptible to infections either by direct contact with infected animals or through their products [14].

EFFECTS OF *SALMONELLA TYPHIMURIUM* ON DIFFERENT SPECIES

Salmonella typhimurium evades the immune system of its host by first being taken up by the epithelial layer of the small intestine and replicating within, specialized vacuoles which causes gastroenteritis in human and other mammals. The PH level within these vacuoles that are produced as result of engulfment induces the production of bacterial gene products required for its survival in the epithelial cell of phagocytes [6]. Moreover, as with all Gram negative bacteria, the outer membrane contains lipopolysaccharides (LPS) which contains lipid A endotoxin. Once this is released it can cause shock, a condition that creates on excessive immune reaction that can be fatal [15].

Cattle: - *S. typhimurium* is the principal causes of clinical salmonellosis in cattle. The disease generally occurs when stress factors are involved. In adult cattle occurs sporadically and begins with fever and the appearance of blood clots in the feces, followed by profuse diarrhea, and then drop in body temperature to normal. Signs of abdominal pains are pronounced. Abortion is common, the diseases may be fatal within a few days or the animal may recover. Whereas in calves it usually acquires epizootic proportions they are more susceptible with high mortality than adults. Septicemia and death are frequent in new borne [16].

Sheep and Goats: - *S. typhimurium* is of the most common serotype that causes gastroenteritis in sheep and goats[18,]. In sheep the disease manifested as acute enteritis, hemorrhagic and necrotic typhilitis and the infection is established in mesenteric lymph nodes and liver. In early stages of outbreaks there may be some case of septicemic form, whereas in goats there is per acute septicemia in new borne and acute enteritis [9].

Swine: - in pigs, the disease varies widely and, although all forms occur in this species, there is often a tendency for one form to be more common in any particular outbreak. Pigs with acute enteritis are usually infected with *S. typhimurium*. A syndrome of rectal stricture also occurs in feeder pigs. In the acute form there is also a tendency for pulmonary involvement to occur, but the main features of the disease is enteritis with pneumonia and occasionally encephalitis presents as only secondary signs. Nervous signs and cutaneous discoloration as described in the septicemia form may also be present [10].

Horse: - the disease in horse usually occurs in a single animal and sporadically. However, the outbreaks do occur in foals, in groups of horses recently transported and in horse hospitalized in veterinary clinics. Once the systemic infection has been established, salmonellosis as a disease can develop and manifested as septicemia, enteritis, abortion and a group of localization in various tissues as result of bacteremia [9].

Table 1: Species affected and possible effects by *Salmonella typhimuriun*

Species	Possible effects
Cattle	Enteritis or Septicemia
Sheep and Goats	Enteritis or Septicemia
Pigs	Enteritis or Septicemia
Horse	Enteritis or Septicemia
Humans	Food Poisoning

Source: [2]

METHODS OF STUDYING DIVERSITY OF SALMONELLA TYPHIMURIUM

Molecular Methods: Genotyping of *salmonella* is becoming an increasingly important epidemiological tool that aids in the identification of source of infection during out break investigations, detection of cross transmission, recognition of particular strains and monitoring intervention strategies. Among genotyping method, PFGE is considered the standard method for DNA fingerprinting in *salmonella* and other food borne pathogens, and a system for differentiating epidemic strains form endemic ones has been proposed. *S. typhimuriun* has been considered very clonal and most Genotyping methods including PFGE do not have the discriminatory power to differentiate within phage types. In the last decade, other methods such as AFLP and Repetitive extragenic palindromic polymerase chain reaction (Rep-PCR) have been developed. For ultimate salmonella strain discrimination, an integrated approach using a highly discriminatory genotyping method together with phenotypic approaches is indispensable [3].

Molecular methods with high discriminatory powers are essential to differentiate among bacterial isolate of colonal descent such as strains of *S. typhimurium*. Therefore, there are three genotyping methods: PFGE, AFLP, and Rep-PCR. These methods are useful to characterize food borne pathogens. PFGE has been limited discriminatory power for some *Salmonella*samovars such as *enteritis*. AFLP has been used since 1995 in eukaryotic and prokaryotic organisms and has gained acceptance in recent years as useful tool for the discrimination of *Salmonella*. It should also be noted that AFLP and Rep-

RCR have a relatively lower reproducibility than PFGE. This is mainly since these two methods employed PCR as one of the steps in the genotyping process. The use of PCR increase likelihood of nonspecific band amplifies action due to potential contaminants that in turn affects the reproducibility [3].

AFLP has found to have the highest discriminatory index of 0.939 with no noticeable difference in reproducibility and with its advantage in high throughput and resolution we employed AFLP as animated of choice to subtype the *S. typhimurium* strains [3].

Phenotypic Method: In this study, two important phenotypic methods are used for the epidemiological investigation of *S. typhimurium* strains. These are Antimicrobial susceptibility testing and phage typing [3].

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility test isperformed on Mueller Hinton agar by disc diffusion method. The antibiotic discs areplaced on the surface of an agar plate previously seeded with a standard amount of the organism to be tested. The plates were incubated at 37°C for 18-24hours**.** Subsequently; the plates were examined for the development of zone of inhibition around the discs. The diameter of the zone of inhibition ismeasured using calipers in mm to the nearest millimeter and interpreted as Susceptible, intermediate or resistance according to the NCCLS values [17].

Antimicrobial susceptibility testing is also performed by using the Vitek Jr. Semi-automated system (Biomeneux: Hazelwood. Mo USA) Using break Points panels. Each isolates isfirst tested against a panel of antimicrobial agents (e.g. ampicillin, tetracycline, chloramphenicol, gentamicin etc). Theisolates with intermediate MIC break points aregrouped with susceptible organisms [3].

Phage Typing: Phage typing of *S. typhimurium*is still a useful tool for surveillance and outbreak investigation. Phage type has for decades been useful as a phonotypical, definitive method for epidemiological characterization of *S. typhimurium*. The system recommended by the world health organization (WHO) collaborative center for phage typing of salmonella has, however, become rather complex, and there arechallenges of sufficient standardization of the interpretation of lysis results to make sure the same strains is assigned to the same phage type in different laboratories [3].

Even though molecular typing method will replace phenotypic characterization methods in the future phage type will remain for some time a useful tool to strength global salmonella surveillance.In demark, phage typing as described the world health organization (WHO) that collaborate center for phage typing of *Salmonella* (Health protection agency (HPA), Colindale, United Kingdom's) has been applied for surveillance of *S. typhimurium* in humans food and food production animals [18].

Phage type provides a rapid, accurate, and cheap method of investigation of S*almonella* strain for epidemiological use. Salmonella strains with in a particular serovars may be differentiated in to a number of phage types by their pattern of susceptibility to lysis by set of phages with different specificity. The phage must have awell defined propagation strains that allow reproducible discrimination between different *Salmonella typhimurium* strains. Different schemes have been developed for this serovar in different countries. More recently, the extended phage typing system of Anderson (England) that distinguishes more than 300 definitive phage types /DTS/ has been used worldwide in Europe, the united states, and Australia [9].

Genotypic Methods of Studying Salmonella Typhimurium: Amplified Fragment Length Polymorphism (AFLP): It is anew and novel technique for DNA fingerprinting. The technique involves three steps:

- Restriction of the DNA and ligation of oligonucleotide adapters.
- Selective amplification of sets of restriction Fragments.
- Gel analysis of the amplified Fragments.

The AFL technique is based on the selective PCR amplification of restriction Fragment form the total digest of genomic DNA. PCR amplification restriction Fragment is achieved by using the adapters and restriction site sequence as target sites for primer annealing. The selective amplification is achieved by primers that extend in to the restriction Fragments, amplifying only those fragments in which the printer extensions match the nucleotides flanking the restriction sites. Using this method, sets of restriction Fragments may be visualized by PCR without know- ledge of nucleotide sequence. The method allows the specific co-amplification of high numbers of restriction Fragments. The number of fragments that can be analyzed simultaneously however, is dependent on the resolution of the detection system [19].

It has higher reproducibility, resolution, and sensitivity at the whole genome level compared to other techniques, but it is also has the capacity to amplify

between 50 and 100 fragments at one time. In addition, no prior sequence information is needed for amplification [20].

Pulsed Field Gel Electrophoresis: Pulsed field gel electrophoresis is a technique used for the separation of large deoxyribonucleic acid (DNA molecules) by applying an electric field that periodically changes direction to gel matrix. It may be used for genotyping or genetic fingerprinting. It is commonly considered a gold standard in epidemiological studies of pathogenic organisms [21].

PFGE is essentially the comparison of large genomic DNA fragments after digestion with restriction enzymes. Since the bacterial chromosome is typically a circular molecule, this digestion yields several linear molecules of DNA. The basic concept of interpretation of this method is the following. If one is comparing two strains that are clonal (i.e. the same strain), the sites of which the restriction enzymes act on the DNA and the length between these sites would be identical. Therefore, after digestion of the DNA and electrophoresis through an agarose gel, if the DNAbanding pattern between any two isolates is identical, then these isolates are considered the same strain. If the DNA banding patterns between any two isolates is not identical then at which the restriction enzymes act on different; thus the DNA binding patterns will be different [22]. *Repetitiveextragenic palindromic polymerase reaction***:** Repetitiveextragenic palindromic-PCR is one of PCR based molecular technique is used to discriminate among bacterial species, serotypes and strains. It is less time consuming and more suitable for analyzing large numbers of bacterial strains in human population. Repetitive extragenic palindromic-PCR targets the highly conserved, interspersed, repetitive elements found at several sites within prokaryotic and eukaryotic genome [23]. The conserved region that lies close to the repeated elements differs according to size, thus producing fragments of varying length, evident via agarose gel electrophoresis [24]. The fragment size provides a distinct fingerprinting profile for the organism, which forms the basis for band comparison [23].

Antimicrobial Resistance of Salmonella Typhimurium: Multi–drug resistant *Salmonella typhimurium* DT104 was first isolated form a human case of salmonellosis in the UK as early as 1980. Since then it has been isolated form humans and other source including food producing animals around the world. It has become a worldwide public health concern. *Salmonella typhimurium* DT104 first demonstrated atypical pattern of penta-resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide and tetracycline (ACSSUT) but it has more recently displayed additional resistance to other antimicrobials like Quinolones in which the mechanism of decreased susceptibility of Fluoroquinolones in DT104 isolates involves point mutations in the Quinolones resistance determining regions (QRDR) of the target gene [25,26]. Multidrug resistant *Salmonella typhimurium* DT104 has also been the first or second most common *Salmonella*serovar reported from human and food producing animals in Canada. The molecular and antimicrobial resistance diversity among *Salmonella typhimurium* DT104 strains wasisolated from different animal source including cattle, poultry and swine. Also diversity in antimicrobial resistance and genotype of DT104 strains isolated from pigs in slaughter houses has been studied recently [7]. Generally, causes of antimicrobial resistance include Plasmid mediated, in industrialized countries has been the over use of antimicrobial in animals feeds as a growth enhancer, where as in developing countries may be self-medication [2].

Prevention and Control: In order to prevent food poisoning caused by species of the genus *Salmonella*, attenuated *Salmonella* vaccine strains have been developed by introducing mutations in to the bacterium that diminish its overall growth. Several strains have been attenuated /deactivated or killed) through the introduction of autotrophic nutritional defects, which slow the growth of the bacterium by preventing it from synthesizing needed biomolecules such as amino acids [8].

Unique control methods are presently not available for *Salmonella typhimuriun* DT104 because little is known about the epidemiology of DT104. The same methods that are effective against other types of *salmonella* Species i.e. good production practices should be control harborage and transmission of DT104. These generally rely on like, companion animals, and wild life; thewide spread decreasing exposure and increasing immunity of susceptible hosts. *S. typhimurium* DT104 has been isolated from a wide range of species including distribution of DT104 makes effective controls potentially more problematic than the control of *S. enteritidis* phage type 4. Of particular importance form a control point of view in cattle identifying clinically ill hard as source of asymptomatic shedders may be one beneficial control measure to minimize the spread of DT104. Flock or herd exposure is decreased through cleaning and disinfecting premises, controlling rodents,

and minimizing contamination of food. Cleaning and disinfesting premises and controlling rodents are most effective in closed environments [8].

Research to control salmonellosis has focused on the use of bacterins to increase immunity. Davies reports that killed bacterins were effective in controlling *S. typhimurium* in cattle [27].

Controlling Salmonella species, in humans relies on decreasing exposure through hygiene processing of products and proper preparation and storage of cooked products. Specific measure include thoroughly cooking foods of animal origin, avoiding cross contamination of other foods, avoiding consumption of unpasteurized dairy products, and educating food handlers. Even in the absence of adequate control methods in animals, instituting control methods at food proportion can still minimize the number of human outbreaks [28].

Public Health Importance of Salmonella Typhimurium: Salmonellosis is globally one of the major foods borne infection in humans. It is a real or potential problem in all areas of the world. It occurs both in sporadic cases and out breaks affecting a family of several hundreds or thousands of people in a population. Food borne salmonellosis in humans continues to be a major public health problem in many countries. In developed countries it is an important food borne disease and accounts for the majority of all out breaks of such disease cases, hospitalizations and death, where the causative agent is identified. It is difficult to evaluate the situation of this disease in developing countries because of lack of epidemiologic surveillance data but epidemic outbreaks are known to occur [16].

The incidence of human salmonellosis has increased during the last decades. This increase has been associated with the spread of *Salmonella* in animal production (meat, milk pork, poultry eggs and egg products), leading to frequent occurrence of salmonella in food for human consumption. Although nearly all Salmonella serotypes are potentially pathogenic to man the two serotypes, *S. typhimurium* and *S. enteritidis*, predominate primarily. This fact reflects an enhanced potential in the serotypes to spread and persist in modern food of animal production systems [20]. *Salmonella*likes the intestinal tracts of humans and her animals, including birds. *S. typhimurium*is usually transmitted to human by eating food contaminated with animal faces, contaminated foods are often of animal origin but all foods including vegetables may become contaminated. Many raw foods of animal origin are frequently contaminated but fortunately through cooking kill the agents. Contributing factors in episodes of food borne salmonellas include slow cooling of decontaminated cooked foods, inadequate cooking of raw food ingredients, improper hot storage of decontaminated cooked foods, cross contamination of prepared foods through faulty handling of raw ingredients, or use of poorly sanitized equipment. Although use of improper holding Temperatures remains the single most common fault in food handling practice, infected food handlers can also play a detrimental role in outbreaks [29].

The incubation period in human is about 6-72 hours and the main symptoms are diarrhea, abdominal pain, vomiting, nausea, mild fever, anorexia, headaches and malaise. The infectious does is said to be from 100 up to10, 000 bacteria's. Less than 100 bacteria may cause illness in young children and in persons who are immunocompromised. Enteritis infection can also occur concurrently with immune deficiency virus (HIV) infection and is one of the common complications of acquired immune deficiency syndromes (AIDS) [30].

Although *Salmonella* infection cause disease, the majority probably leads to sub- clinical encase resulting in healthy carrier state with intermittent excretion of salmonella in the faces. Whether a human develops disease following ingestion of salmonella depends on the dose of organisms, the serotypes of *Salmonella*, and up on specific and nonspecific immunological factors [31].

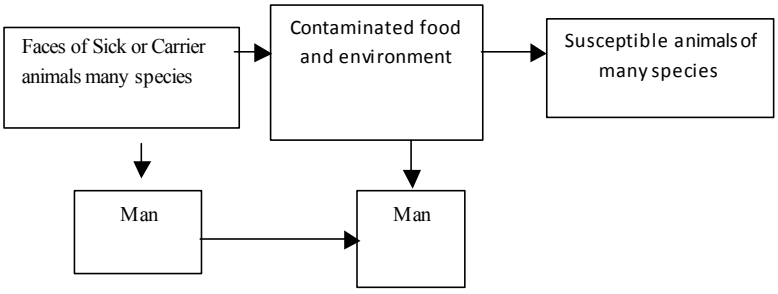

Fig. 1: Mode of transmission of *S. typhimurium* [16]

Human salmonellosis is wide spread in young children in elderly citizen frequently afflicted with underling chronic diseases, and is particularly susceptible to infection. Transmission of the infectious agent occurs primarily between humans, form various animal and environment. Changes in eating habits, mass catering and increased internal movement of foods and food ingredients have certainly contributed ofthe observed increase in food borne salmonella out breaks [1].

CONCLUSIONS AND RECOMMEDATIONS

Epidemiologically *Salmonella typhimurium,* which is distributed throughout the world, affect a wide host range. The mode of transmission is complicated and great number of animals, birds and reptiles are responsible for the maintenance the chain of infection. Food stuffs, birds, bedding, flies, rodents, sewage, soil, water and carrier state of animals are the main sources of infection. The use of molecular tools and techniques has paramount importance in the diagnosis and investigation of food borne diseases due to *Salmonella typhimurium*. Multiple drug resistance (MDR) grouping have emerged and now a day's isolates pertaining to them are more frequent than drug susceptible. The main cause of antimicrobial resistance in industrialized countries has been the over use of antimicrobial in animals feeds as a growth enhancer, where as in developing countries may be self-medication. Unique control methods are presently not available for *Salmonella typhimuriun* DT104 because little is known about the epidemiology of DT104.

Therefore, based on the above conclusions the following recommendations are forwarded:

- Hygienic measures and procedures should be followed in all food chain system from farm to fork.
- Awareness creation through Radio, TV, Magazines and other Communication Media should advocate the hazard related to consumption of undercooked foods of animal origin.
- Further study dealing on identification of the emergence of new pathogenic and drug resistant strain of Salmonella organism should be conducted.
- Drugs should be used according to the direction of physicians' and veterinarians in order to avoid antimicrobial resistance.
- A strong collaboration between veterinarian, physicians and environmental health science professional should be present.
- The application of molecular biology techniques in disease investigation and prevention should be supported by the government in developing countries.
- Sciences based partnership between developing and developed countries should be present.

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