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Review on Clinical Evaluation of Herbal and Allopathic Drugs for the Treatment of Infective Diarrheial Diseases (Shigellosis)

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ABSTRACT

Dysentery is characterized by intense abdominal cramps accompanied by frequent and intense diarrhoea with bloody mucous stool. The word dysentery has been derived from Greek words dys and enteron meaning "bad bowels". Micro-organisms that cause gastrointestinal diseases must survive the harsh acidic environment of the stomach. Shigella spp. and Escherichia coli O104:H4 are gastrointestinal bacteria that do not form special resistant structures but can survive at pH 2.5 for at least 2 hours. Studies with human volunteers have shown that when as few as 10-500 Shigella are ingested, they can survive passage through the stomach and cause dysentery. Over the last three decades, multidrug resistant Shigella was responsible for widespread epidemics of Shigellosis on the Indian subcontinent and in other developing countries, leading to high mortality. As Shigellosis is highly contagious it is crucial to develop a rapid method for eradication of the pathogen in order to limit and control outbreaks.

Keywords: Culture Media, Dysentery, Multi Drug Resistance, Shigellosis, Shigella.

INTRODUCTION

Dysentery:

The term dysentery is specifically used for the passage of stool with pain and cramps. Dysentery is of two kinds one is limited to rectum, while other is confined to transverse colon, which is in contact or proximal to the rectum. Difference in both is obvious as dysentery occurs due abrasion and muco-purulent discharge. There is no discomfort found in the rectum, tenesmus is less, excretion is possible by least tenesmus and patient remains asymptomatic. In rectal dysentery, defecation

is painful with small fecal matter and tenesmus and patient gets fatigued easily [1].

Types:

Waseem Ahmed Azmi [2] described the types of dysentery as under:

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Acute Dysentery/Zaheer Had:
Initial stage, stool yellow colored accompanies cramps, mucus watery diarrhea, anorexia, later on other symptoms appear like recurrent feeling of defecation, straining tenesmus during defecation, stool with blood, mucus, membranous scales and obstruent matter, in serious conditions patient use to defecate 10- 40 times with extreme pain and cramps, heaviness in rectal area, colonic pain that increases on palpation, fever, skin hot and dry, insomnia, facial and eyes look reddish, dysuria with reddish coloration and burning micturation, pulse brisk and hard, lethargy.

Chronic Dysentery/Zaheer Muzmin:
Less cramps, alternate situations of constipation and diarrhea, tongue reddish and lustrous sometimes cracks, diarrhea usually somewhat watery along with acrid smell, membranous scales, pus and blood and facial expressions lethargic and yellowish.

Real Dysentery/Zaheer Sadiq:
Symptoms similar to acute dysentery and some scholars claim to be the same. In diarrhea bilious/acidic phlegm being excreted along with blood. In case of bilious matter there will be a needle like pinching pain, dominant hotness and thirst, pulse will be brisk and continuous. Diarrhea accompanies blood, bilious matter and ulceration. Cold temperamental therapies will comfort the patient, phlegmatic matter will accompanies mucus, flatulence without ulceration, pulse will be feeble.

Unreal Dysentery/Zaheer Kazib:
Intestinal obstruction results in painful and straining defecation. There will be tenderness all over, continuous pain scanty stool with mucus and blood only on straining like the residue of goats, due to flatus will produce cramps.

Bacillary Dysentery/Zaheer Asawi:
It is colonic dysentery, sometimes inner portion of intestinal epithelial lining gets affected. In this case intestinal membrane becomes edematous that results in tissue death. These membranous scales are excreted out in stool.

Amoebic Dysentery/Zaheer Amoebae:
Organisms affect the intestinal lining by activating the proteolytic fermentation. That can lead to inflammatory and edematous conditions. Intestinal elasticity is reduced eventually resulting in ulceration and stool accompanies blood and scales.
Inflammatory Dysentery/Zaheer Warmi:
Intestinal and rectal swelling along with heaviness, pinching pain and cramps. Tenesmus is also present and strain on bladder lead to dysuria, fever, and pulse dicrotic.

Infectious Dysentery/Zaheer Wabaiee:
Spread by person to person. Skin papules, itching, inflammation are common. Usually patient goes to syncope after every defecation.

Zaheer Sifli:
Abdominal heaviness, pain and cramps. Dry chick beans sized stool. History of intake of dry foods and medicines.

Cold Temperamental Dysentery/Zaheer Bardi:
History of taking cold foods and other stuffs. Patient feels to be relieved by taking hot temperamental therapies.

The humor that can produce dysentery is the one with caustic (escharotics) property. Transverse colon and rectum either have been exposed to laceration or about to be lacerated under effect of the said humor. This humor exhibits the activity of caustic or escharotics property which leads to increase sensitivity to the effected parts. If the abrasive action is restricted to transverse colon then dysentery will be less severe and it will not cause strain in the distal intestinal portion.

Dysentery is the ulceration of transverse colon and it becomes worse when accompanying intestinal wounds. According to Galen, in dysentery and colic pain there is intense pain without diarrhea. He stated this just to review the assimilation of Maghas (colic).

Nevertheless there is a immense difference between Coloni (colic pain) and Maghas (colic). Likewise between Coloni (colonic pain) and dysentery, due to the fact that in zaheer (dysentery), patient has to strain to defecate. Whereas in coloni(colic pain) there is no stool on defecation. Maghas(colic) that is the flatus moving around along with mucus giving the sense to defecate, which is either less or none in quantity.

Zaheer (dysentery) symptom is that mukhati shai (mucous scales) are secreted and the anal area is effected by intense pain with Lazaa (burning), sozish (irritation), back khichaoo(stress) and tamaddud (distention). Intestinal acrid smell ulceration with low heat and smelly sediment like stool are the sign and symptoms of Zaheer.

In long incubation period, dysenteric diarrhea smelly substance is secreted. But in dysentery no smelly substance is secreted because in dysentery ulcer is located near the rectal region. While in dysenteric diarrhea ulceration is distant from rectal area. Dysentery mostly occurs in phlegmatic humor, because tahaddar is the result of putrid phlegm in the amaa mustaqeem (transverse colon). Due to putrification patient feels to defecate frequently. In zaheer (dysentery) there is intense spasm and cramps because phlegm sticks to intestinal wall and it needs to be defecated by force as compared to bilious humor. Bilious intestinal ulcerations are acute and lethargic condition occurs due to its excess heat.

Intestinal ulceration due bilious irritation and heat persist and is of 2 weeks duration. Salty phlegm ulceration is confined to 30 days whereas melancholic ulceration occurs for 40 days or more. If ulceration is confined to rectal area without abdominal cramps, then it is the sign of dysentery. If there is secretion of mucus and flatus then it is assumed that ulceration is in the small intestine. But if the mucus and flatus is in excess and thick in consistency, then it is the sign that ulceration is in large intestine. Ulceration in upper intestinal tract is associated with intense pain at epigastric region. The secretion of mucus along with fatty substances is the indication of ulceration of lower intestinal tract. Dysentery patient feels to defecate frequently. Secretion of mucus substances before stool is because of ulceration in rectal region, secretion of mucus after stool is the indication of healing [3].

Micro-organisms that cause gastrointestinal diseases must survive the harsh acidic environment of the stomach. Shigella species and Escherichia coli are gastrointestinal bacteria that do not form special resistant structures but can survive at pH 2.5 for at least 2 hours [4].

In bacterial infections, disease is a result of microbial growth in body tissues rather than of the ingestion of food and drink already contaminated by preformed toxins resulting from microbial growth. Bacterial infections such as salmonellosis and shigellosis usually have longer incubation periods (12 hours to 2 weeks) than bacterial toxicity reflecting the time needed for the microorganism to grow in the host [5].

When the pathogen reaches the colon, it translocate through the epithelial barrier by way of the M cells that overlay the solitary lymphoid nodules [6].

M cells count is enhanced throughout chronic intestinal inflammation detecting apoptosis as well. There is a correlation seem to be in M cell damage and amplifying uptake of pathogens at some stage in intestinal inflammation. Epithelial barrier is busted in the inflammatory process for the M cells to play a vital function [7]. When Shigella get in touch with the principal M cells it effects the underlying macrophages moreover provoke cell demise. The infected macrophages liberate great quantity of interleukin-1 that lastly escort to a powerful inflammatory response [8]. Afterwards bacterium liberated from the macrophages enters epithelial cells through the basolateral surface as a result of membrane rumpling furthermore macro-pinocytosis. Phagocytic vacuole engulfs bacteria, so as to disrupt the membrane to break out into the cytoplasm

resulting in multiplication furthermore travel through provoking actin polymerization at one pole of the bacterium permitting intracellular spread inside the cytoplasm and also to neighboring epithelial cells. As a result of bacterial infection epithelial cells are bring forth proinflammatory cytokines, additionally endorsing restricted colonic inflammation. Accordingly for Shigella species to cause infection unremitting intra- as well as intercellular dispersal is crucial as a causative factor for bacillary dysentery [9, 10].

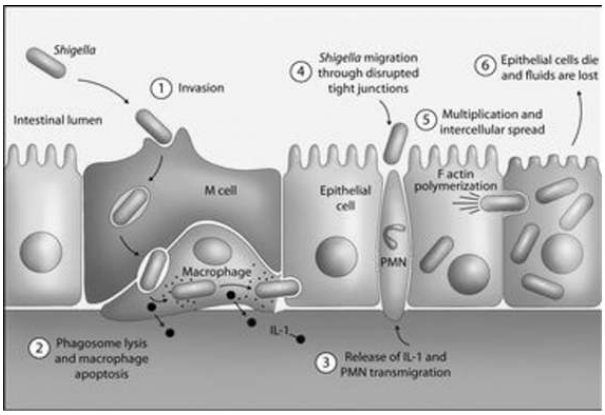

Figure 1: Pathogenesis of Shigellosis

The load of shigellosis was recorded in Asia from available data, which signify that the yearly figure of Shigella incident along with fatality in Asia was anticipated to be 91 million. Shigella flexneri is the most frequent serotype next is Shigella sonnei. At the time of epidemics the highest level of fatality rate recorded in Bangladesh which was about 75000 deaths effecting kids other than the epidemic areas in non epidemic ones the death rate reported to be 35000 on yearly basis. In broad-spectrum, equally the occurrence as well as the casualty rates is maximum effecting extremely young children and the aged. One of the case-control study exemplified the epidemiological patterns of diarrhea with blood in countryside of Western Kenya stated that 80% of the entero-coccal microbes detected found to be Shigella spp, the concerning strain was around 49% Shigella flexneri [11-20].

Classification:

The type Shigella is strongly interrelated to the group Escherichia so as to fit into the family Escherichieae. Shigella organism though is not affiliated to the typical gastrointestinal flora as well as each Shigella species is able to lead to bacillary dysentery. The type Shigella was given name subsequently after the Japanese Microbiologist Kiyoshi Shiga, who isolated the original constituent of the class in 1896 from the stool of a person effected from dysentery in Japan. It was then called Shigella shigae refers now as Shigella dysenteriae serotype 1. The different Shigella spp. such as Shigella flexneri, Shigella sonnei and Shigella boydii were thus illustrated by Flexner in 1900, Sonne in 1915 and Boyd in 1931 respectively [21].

Antigenic Structure:

All Shigella species possess O antigens and certain strain may possess K antigens. Shigella K antigens when present interfere with the detection of the O antigen during serologic grouping. The K antigen is heat-labile and may be removed by boiling the organism in a cell suspension. On the basis of somatic O antigens Shigella can be subdivided into serotypes. This is carried out by agglutination tests with absorbed specific antisera [21]. Based on antigenic structure and biochemical reactions, Shigella organisms are divided into four serogroups corresponding to the following species:

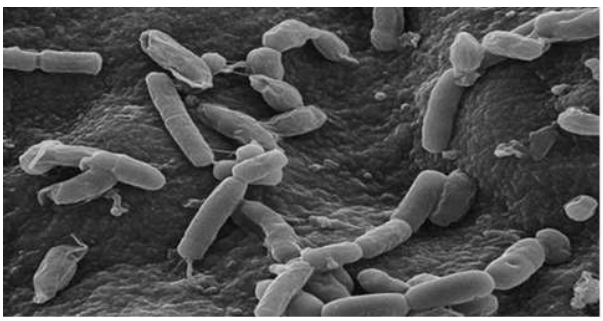

Figure 2: Shigella gram negative rods

Clinical Signs and Symptoms:

Clinical signs and symptoms of the disease initiates after 24-48 hours of entry of the pathogen. Shigellosis is described by recurrent course of little amount of stools mostly of blood, mucus and pus go along with

fever in addition to stomach cramps. Fecal blood, mucus and pus cells in are the indicators of colorectal inflammation. All the indicators are too familiar in infections originating by Campylobacter, Salmonella and Entamoeba histolytica nevertheless turn out self limited disease that is hardly ever as serious as Shigellosis. Other than fecal blood patients with dysentery frequently have rectal pain, weakness, malaise and anorexia. Conversely in a few cases, Shigella results in acute non-bloody diarrhea that cannot be notable clinically from diarrhea caused by further enteric microbes. Rigorous cases may grow to be life threatening and take part to substantial mortality. Disease with severity of infection possibly will excrete exceedingly 20 dysenteric stools per day. In other words dysentery is said to be daily loss of 200-300 ml of serum protein in the feces that aggravate undernourishment as well as development. Chances of risk of shigellosis and substantial mortality increases due to diminution of immune system. Diseased indicators generally continue for at least 10-14 time duration or longer [22-24].

Laboratory Diagnosis:

Hematology:

The total leukocyte count is frequently within reference range. Nevertheless in a few cases, leucopenia, Anemia and thrombocytopenia may occur.

Bacteriology:

Stool Examination:

Stool microscopy is distinctive for instance stool texture (liquid, semisolid or formed stool) and unusual constituent (mucus, blood, non-bloody stools) is the chief characteristic in resolving the probable microbe resulting in the diarrhea. In addition, usual microscopic examinations for the existence of erythrocytes and leukocytes are significant feature to ascertain the judgment of dysentery. Fecal matter that is fresh with unstained suspension is observed directly through microscope. Infiltration of the polymorph nuclear leukocyte as well as erythrocytes in the fecal secretion resulting from assault of the intestinal lining and the consequential inflammatory reaction. Either fecal erythrocytes or leukocytes are demonstrable in the stool in around 70 % of Shigellosis cases while evenly presence of fecal erythrocytes and leukocytes are identified in about 50 % of cases. Nevertheless verification of laboratory findings is accomplished through stool culture.

Stool Culture:

Exact conclusion of Shigella infection is through isolation of the pathogen from fecal sample as well as serotyping the isolate. Culture is as well necessitate for antibiotic susceptibility testing. Prior to commencement of antibiotic therapy fresh stool samples are taken from patients are favored for microbiological investigation since the option of growth of the organisms is highly developed. Shigella is frequently isolated from stool sample via culture methods afterwards recognition by means of biochemical tests along with serological agglutination evaluation.

Preferably samples that cannot be examined within the limited time period should be stock up at 4°C in transport medium (buffered glycerol saline or Cary-Blair medium). Separation in conjunction with recognition of Shigella can be significantly improved whilst most favorable laboratory media as well as methods are applied. As Shigella is a extremely fastidious pathogen suitable collection, quick carrying to the laboratory and swift plating of the sample are essential in favor of victorious results. Similar circumstances are frequently complicated to accomplish, particularly in developing nations. Consequently, the resurgence rate of Shigella is typically less. Although as a consequence Shigellosis stay undiagnosed in a majority of patients [25].

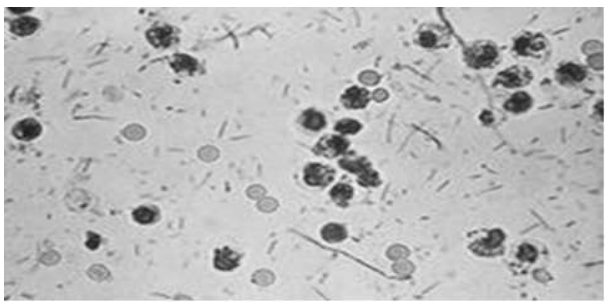

Figure 3: Shigella in stool specimen

Inoculation of Selective Media:

Separation of *Shigella* typically includes an primary streaking for isolation on differential or selective media with aerobic incubation to restrain the growth of the anaerobic normal flora. Stool specimens or rectal swabs are inoculated onto primary isolation media like MAC, DCA, XLD HEK otherwise SS agar.

Culture Characteristics:

Shigella is a facultative anaerobe bacterium and is able to grow at temperature ranging from 12°C to 48°C (optimum 37°C) at a pH range of 5.0 to 7.3. Generally, termination of *Shigella* are enhanced with increased in temperature, decreased in pH and increased in NaCl concentrations. The organism is acid resistant and can easily pass the gastric acid barrier. The common selective/differential agar media used for the recovery of *Shigella* are MacConkey (MAC), Xylose Lysine Deoxycholate (XLD), Hektoen (HEK) and *Salmonella-Shigella* (SS) and Deoxycholate Citrate Agar (DCA). It has typical non-lactose fermenting characteristic colonies on lactose enriched media such as on MAC, DCA and SS agar. *Shigella* is resistant to bile salts and this characteristic is usually useful in the selective media. Colonies on the MAC and DCA agar appears to be large 2-3 mm in diameter, translucent and colorless (non-lactose fermenting). Whereas on the XLD agar colonies appear to be much smaller 1-2 mm diameter and red in color as lysine is decarboxylated producing alkaline end products which raises the pH and cause the agar to turn into deep red color. *Shigella* does not produce hydrogen sulphide on the XLD, HEK and SS agar [21].

Biochemical Reactions:

On the basis of biochemical reactions and antigenic structure *Shigella* is classified into 4 groups (A, B, C, D). *Shigella* are methyl red positive and negative for Voges-Proskauer reaction, citrate and malonate utilization, lysine decarboxylation, urease and H_2S production, gelatin liquefaction, phenylalanine deamination and are unable to grow in the presence of KCN. All the *Shigella* Species produce acid from glucose and with the exception of *Shigella dysenteriae* from mannitol. *Shigella dysenteriae* serotype 1, *Shigella flexneri* serotype 6 and *Shigella sonnei* are always indole negative. *Shigella sonnei* is a late lactose fermenter and colonies growing on MAC or DCA medium for more than 24 hours develop pink color. Other three species are non-lactose fermenters. Only *Shigella sonnei* forms ornithine decarboxylase. *Shigella dysenteriae* serotype 1(*Shigella shigae*) and many strains of *Shigella dysenteriae* serotypes 3, 4, 6 and 9, *Shigella flexneri* serotype 4a, and *Shigella boydii* serotype 13 which are catalase negative [26, 27].

Figure 7: Colonial Morphology of Shigella sonnei on MacConkey Agar

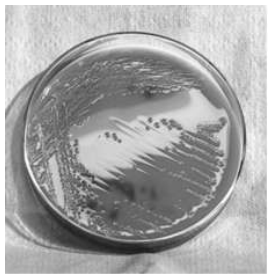

Figure 8: Colonial Morphology of Shigella boydii

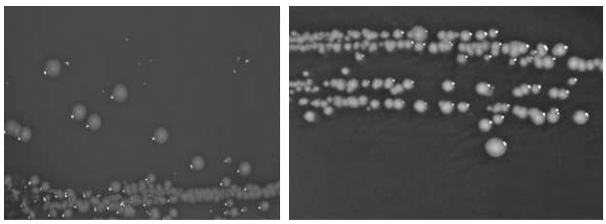

Figure 14 : Colonial morphology displayed by Shigella Spp

Serological Identification:

Serological testing is executed for verification and species categorization of the Shigella isolates. It is accomplished by slide agglutination analysis by means of commercially accessible polyvalent O antigen grouping sera. Though, in few cases, precise serotype recognition is executed via testing with monovalent antisera for serotypes and sub-serotypes identification. All species of Shigella have a characteristic type of O antigen. Agglutination tests

is carried out by applying a clean glass slide by emulsifying a part of the growth from the surface of KIA, TSI or other non-selective agar media in a drop of physiological saline. Colonies from selective media like MAC or DCA are not advised to be used for this reason since it might turn out false negative results. A small drop of polyvalent or monovalent antiserum is merge with the suspension to monitor for the agglutination response. Shigella polyvalent antiserum will agglutinate strains of the equivalent serogroup and monovalent antiserum will agglutinate the specific serotype or sub-serotype. Cultures that react serologically and show no contradictory outcome in the biochemical screening analysis is stated as positive for Shigella.

Other Diagnostic Techniques:
DNA Based Method:
Current progress in molecular biology have established novel findings for the quick as well as susceptible judgment of microbial infections by identifying the existence of pathogen definite DNA series in laboratical samples without the requirement for culturing the bacteria. Polymerase Chain Reaction (PCR) signifies an influential means in studying bacterial infections to amplify the target DNA through a million fold in fewer than 2 hours. PCR gives information on the existing infection condition and is autonomous of the host's immune proficiency. Nevertheless, restrictive concern that prevents DNA technology from common use in the investigative laboratory is that, it is costly to execute and necessitate classy tools which might not be available in the developing countries. Additionally the existence of PCR inhibitors in the intricate specimens like fecal matter, food as well as in culture media can inhibit augmentation of the marked genes, hence, restricting the worth of the PCR performance in analysis.

Immunological Assay:
The majority of quick techniques include immunological assays for precise detection of microbial infections. During the assessment these techniques utilize antibody or antigen identifying merely single or a cluster of bacteria. Current progress in immunoassay technology makes recognition quicker, further suitable, additionally receptive as well as precise as compared to conservative culture routine. The exceedingly definite combination of antibody toward antigen in addition, the minimalism and adaptability of this response has assisted the mean of a diversity of antibody assess. There is quite a lot of fundamental layout of antibody based assays like latex agglutination, immunodiffusion and enzyme immunoassays (EIA) format [28].

CONCLUSION_MULTIDRUG RESISTANCE:

In one of the study, regarding antimicrobial resistance of Shigella spp., it has been cited that 27 % of the entire Shigella isolates exemplify full sensitivity, although 73% were extremely resistant to at least single antibiotic. In general, 72%, 57%, 56%, 55%, 41%, 34% and 8% of Shigella variety were resistant to streptomycin, tetracycline, ampicillin, chloramphenicol, trimethoprim, trimethoprim-sulphamethoxazole and kanamycin correspondingly. Each strain was susceptible to ciprofloxacin, ceftriaxone and amikacin excluding three strains of Shigella flexneri serotype 1b with intermediate susceptibility to amikacin. Although ten Shigella dysenteriae serotype two were resistant to streptomycin whereas Shigella dysenteriae serotype six as well as Shigella boydii strains were completely sensitive. During the time period 1997-2000 resistance concerning Shigella flexneri to ampicillin, chloramphenicol, streptomycin in addition to tetracycline maintained to be high with barely negligible instability. This picture is depicted mostly in countries like Israel, Northeast Brazil, Hong Kong, Egypt. Perhaps persistent resistant strains are due to constant use of antibiotics in the cure of shigellosis [29- 32].

In Indian subcontinent along with other developing nations more than the preceding three decades, multiple antibiotic resistant Shigella dysenteriae 1 was accountable for pervasive outbreaks of shigellosis directed towards increasing death. In 2001-2002 studies from India highlights nalidixic acid resistance in Shigella sonnei in 94-100% of the strains isolated.

The cautious situation of emergence of nalidixic acid resistance along with decrease ciprofloxacin

susceptibility against Shigella sonnei in the regions. This needs further watchful studies and secured use of such antimicrobials to avoid resistance, as this strain is prevailing either in developed or developing nations, so continuous observation is the key to success for its eradication and avoidance of resistance [33].

Possibly due to the fact that this drug was initiated in Gondar just lately. Fluoroquinolones like ciprofloxacin as well as norfloxacin appeared to be used in Gondar in the 1990s as the ideal drugs for the cure of Shigellosis. In Pakistan nalidixic acid continue to be the drug of preference for shigellosis. The drug preserves substantial intracellular amount that is consider to be idyllic for efficiency next to inflammatory diarrheal conditions like shigellosis [34-36].

The extent of the antibacterial activity point out that the crude extract is a prospective resource of bioactive complex that might be functional for the manufacture of novel antibiotics for declining the load of drug resistance as well as expenditure of managing health problems in South Africa. As plants generate a various series of bioactive molecules making them affluent basis of diverse category of drugs, pharmacological as well as toxicity analysis will be obligatory to setup their safety as effective drugs [37-39].

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