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Guidelines for the control of epidemics due to *Shigella dysenteriae* type 1

World Health Organization
Emerging and other Communicable Diseases, Surveillance and Control

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1. INTRODUCTION

Shigella dysenteriae type 1 (Sd1) is an unusually virulent enteric pathogen that causes endemic or epidemic dysentery with high death rates. It is the *only* cause of large-scale, regional outbreaks of dysentery. In recent years, Sd1 has caused epidemic dysentery in Central America, south Asia, and central and southern Africa. An epidemic in Central America from 1969 to 1973 was responsible for more than 500,000 cases and 20,000 deaths. The epidemic in central and southern Africa began in 1979 and has affected at least nine countries. It is likely that most developing countries are at risk of epidemic dysentery due to Sd1.

These guidelines are intended to assist national health authorities, public health officers and health care providers in their efforts to prevent and/or treat Sd1 disease. The text describes the epidemiology, clinical features and management of disease caused by Sd1, and interventions that can reduce both the incidence of Sd1 infections and mortality due to Sd1 disease.

2. ABOUT *SHIGELLA DYSENTERIAE* TYPE 1 (SD1)

Shigella are the most important cause of acute bloody diarrhoea (dysentery). *Shigella* cause dysentery by invading and destroying cells that line the large intestine, leading to mucosal ulceration, a haemorrhagic inflammatory exudate and bloody diarrhoea. Apart from bloody stools, patients with dysentery often have fever, abdominal cramps and rectal pain. In almost half of cases, however, *Shigella* cause acute non-bloody diarrhoea that cannot be distinguished clinically from diarrhoea caused by other enteric pathogens.

Sd1 differs from the other *Shigella* serogroups (*S. flexneri, S. sonnei, and S. boydii)* in three important ways: (i) only Sd1 causes large and prolonged epidemics of dysentery, (ii) antimicrobial resistance occurs more frequently among Sd1 than other *Shigella* serogroups, and (iii) infection with Sd1 causes more severe, more prolonged, and more frequently fatal illness than does infection with other *Shigella* serogroups.

Sd1 disease is most often severe or fatal in young children, especially infants, and in the elderly and the malnourished. Although most patients recover without complications within seven days, persistent diarrhoea may occur occasionally. Other complications of Sd1 infection are haemolytic-uraemic syndrome (HUS), seizures, sepsis, rectal prolapse and toxic megacolon. The case-fatality rate without prompt effective treatment ranges from 1% to 10%.

Infection with Sd1 is most common in overcrowded areas with poor sanitation, sub-standard hygiene, and unsafe water supplies. Refugee populations may be at especially high risk. During epidemics, up to one-third of the population at risk may be infected. Illness tends be seasonal, occurring during hot, wet weather. Seasonality is less pronounced, however, in Africa. Transmission of Sd1 probably occurs mostly through person-to-person contact and through contaminated food and water. The infectious dose is low; the ingestion of as few as 10-100 organisms has caused disease in volunteers.
During dysentery, *Shigella* are excreted in large numbers in the stool (10^6-10^8 bacteria per gram). *Shigella* have been documented to survive in soiled linen for up to seven weeks, in fresh water for 5-11 days, in salt water for 12-30 hours, in dust at room temperature for six weeks, in sour milk for four weeks and in kitchen refuse for 1-4 days. Survival is prolonged at temperatures below 25^{o}C. Freezing will not eliminate the organism, although it may reduce the number that survive.

3. OTHER CAUSES OF DYSENTERY

Aside from Sd1 and other *Shigella*, endemic dysentery is caused by *Campylobacter jejuni*, enteroinvasive *Escherichia coli*, *Salmonella* and, infrequently, *Entamoeba histolytica*.

Enterhaemorrhagic *E. coli* O157:H7 has caused localized outbreaks of dysentery in Europe and North America, usually associated with eating undercooked contaminated beef or drinking raw milk; person to person transmission also occurs. Between 5% and 10% of patients with severe bloody diarrhoea develop HUS. Approximately 20% of persons with HUS die and an additional 30% develop chronic renal failure. A related organism, *E. coli* O157:NM, has caused at least one large outbreak of dysentery in southern Africa. Laboratory techniques for identifying *E. coli* O157 are given in Annex 9.

E. histolytica is an occasional cause of dysentery, especially in young adults, but *does not cause epidemic disease*. Asymptomatic infection with *E. histolytica* is, however, frequent in developing countries, being present in up to 10% of healthy persons. In some epidemics of dysentery due to Sd1, *E. histolytica* was identified and initially thought to be the cause. Because of this incorrect diagnosis, persons with dysentery were treated with anti-amoebic drugs (such as metronidazole), with resulting continued transmission of Sd1 and excess preventable mortality. Finding cysts of *E. histolytica* in bloody stool during an epidemic does *not* indicate that it is the cause of the epidemic, or even that it is the cause of dysentery in an individual patient.

4. PREVENTION OF INFECTION WITH *SHIGELLA DYSENTERIAE* TYPE 1

Infection with Sd1 is spread by direct contact with an infected person, or by eating or drinking contaminated food or water. Preventive measures are summarized below.

4.1 Health education

Health education is the key to public awareness and cooperation. Experienced health educators play an important role in epidemic control. Community groups and service organizations can also assist by disseminating messages through their programmes.

The public must be informed about how infection by *Shigella* is spread and how it can be prevented. Messages should be spread through home visits, health facilities, schools, religious leaders and the mass media. Messages must be carefully prepared, taking into consideration the local terminology, cultural sensitivities, traditions and beliefs. Only measures that it is possible to implement and that have a high likelihood of preventing transmission of the disease should be promoted. Particular attention should be given to the strategies presented below, which are also effective for reducing morbidity and mortality from endemic shigellosis and from acute watery diarrhoea caused by other pathogens, such as cholera. Some examples of public health messages are contained in Annex 1.

4.2 Hand-washing with soap

Hand-washing with soap may be the most effective measure to prevent transmission of *Shigella*; it should be promoted in every family. Hand-washing is particularly important after defecation, after cleaning a child who has defecated, after disposing of a child's stool, before preparing or handling food and before eating.

Hand-washing is practised more frequently where water is plentiful and within easy reach. If possible, water for washing should be stored separately from drinking-water. During an epidemic of Sd1 disease, soap should be provided to those without it. If soap is not available, ash or earth can be used to scrub the hands. Washed hands should not be dried with dirty cloths.

4.3 Breastfeeding

Breastfeeding of infants and young children should be promoted. Infants and children who are breastfed have fewer episodes of diarrhoea or dysentery due to *Shigella*; when these do occur, they are less severe than in those who are not breastfed. This protection is greatest in infants who are exclusively breastfed until 4-6 months of age, but remains significant when breastmilk is given with other foods, even into the third year of life.

4.4 Food safety

Each country should establish adequate controls for the handling and processing of food through a national programme on food safety. Environmental health workers should monitor food-handling practices and be given the authority to stop street sales or close restaurants when their inspections reveal unsanitary practices.

Health education for the general population should stress the following messages concerning the preparation of food for adults, children and infants (see also Annex 2):

- do not eat raw food, except undamaged fruits and vegetables that are peeled and eaten immediately;
- cook food until it is hot throughout;
- eat food while it is still hot, or reheat it thoroughly before eating;
- wash and thoroughly dry all cooking and serving utensils after use;
- keep cooked food and clean utensils separate from uncooked foods and potentially contaminated utensils;
- wash hands thoroughly with soap before preparing food;
- protect food from flies by means of fly screens.

4.5 Safe drinking-water

An adequate quantity of safe water must be available for drinking. Water supplies should be adequate to meet all the needs of a population all year round. It is recommended that a minimum of 20 litres of water per person per day be available. Health clinics and hospitals require 40-60 litres per patient per day. Ideally, no dwelling should be located more than 150 metres from a water source.[1,2] General guidelines for ensuring a safe water supply are given below.

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1 *United Nations High Commissioner for Refugees Handbook for Emergencies*. Geneva, 1982.

2 Cairncross S, Feachem RG. *Environmental health engineering in the tropics: an introductory text*. New York, John Wiley and Sons Ltd., 1983:28-33.

4.5.1 Water supply

Piped water must be properly chlorinated. Recommended chlorine levels for piped water are given in Annex 3. Leaking joints should be repaired and constant pressure should be maintained in the system to prevent the entry of contaminated groundwater.

Where an exposed water source (a river, pond, or open well) is used for drinking-water, it should be protected from contamination by people and animals. This may require that a fence be built around it. Drainage ditches should be dug to prevent storm water and other surface water from flowing into the drinking-water source. Defecation must not be allowed within 10 metres of the water source, and should be downhill, or downstream, from it. Wells should be equipped with a well-head drainage apron, and with a pulley, windlass, or pump. Other water sources should be provided for bathing, washing and other purposes.

Where locally available water is likely to be contaminated, drinking-water should be supplied by tankers or transported in drums, provided it is adequately chlorinated and a regular supply can be ensured. The trucking of water is, however, expensive and difficult to sustain; it is usually considered a short-term measure until a local supply can be established.

4.5.2 Home storage and treatment

Families should be encouraged to store drinking-water in covered containers that are cleaned daily, to keep drinking-water away from children and animals, and to use a long-handled dipper, kept specially for the purpose, to take water from the containers. Another approach is to store drinking-water in a narrow-mouthed container, with an opening too small to allow the insertion of a hand. When the safety of drinking-water is uncertain, it should be chlorinated in the home (see Annex 3) or boiled. Heating water until it starts to boil vigorously is adequate to kill *Shigella* and other bacterial pathogens. Boiled water should be stored in a separate sealed or covered container. Water used for purposes other than drinking need not be boiled.

4.6 Disposing of human excreta

High priority should be given to ensuring the safe disposal of human waste. Sanitary systems appropriate for local conditions should be constructed with the cooperation of the community. Designs for latrine construction in different types of soils and climatic conditions can be found elsewhere.[3] See also Annex 4 for instructions on making a ventilated improved pit latrine.

Health education messages should stress the need for proper use of latrines by everyone, including children. They should also stress the dangers of defecating on the ground or in, or near, the water supply. The disposal of children's excreta in latrines should be emphasized. If children defecate on the ground, the faeces should be picked up, using a scoop or shovel, and deposited in a latrine or buried.

When large groups of people congregate, as for fairs, funerals or religious festivals, particular care must be taken to ensure the safe disposal of human waste. Where there is no latrine, defecation should be performed in marked areas and a shovel provided to bury the faeces.

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[3] Franceys R, Pickford J, Reed R. *A guide to the development of on-site sanitation.* Geneva, World Health Organization, 1992 (ISBN 92 4 154443 0).

4.7 Preventing spread of *Shigella dysenteriae* type 1 in health facilities

The following steps can help to reduce the spread of Sd1 infection in clinics and hospitals:

- provide plenty of water and soap for hand-washing, preferably in easily accessible, highly visible locations;
- wash hands with soap before and after examining each patient;
- ensure that health workers who care for dysentery patients (or other diarrhoea patients) do not prepare or serve food;
- dispose of stools of dysentery patients in a latrine or toilet (if this is not possible, bury them);
- wash and disinfect the clothes and bed linen of dysentery patients frequently.

4.8 Disinfecting clothing and disposing of bodies

Prompt and thorough disinfection of a patient's clothing, personal articles and immediate environment can help to control spread of infection within a family. Effective and inexpensive disinfectants include: chlorinated lime powder, 2% chlorine solution, and a 1-2% solution of phenol. Clothes should be washed thoroughly with soap and water, and then boiled or soaked in disinfectant solution. Sun-drying of clothes is also helpful since direct sunlight will kill Sd1. Utensils may be washed with boiling water or disinfectant solution. The washing of contaminated articles, particularly clothes, in rivers and ponds which might be sources of drinking-water, or near wells, must be prohibited.

Funerals of persons who die with diarrhoea, whether bloody or not, should be held quickly and close to the place of death. The washing of dead bodies and the preparation and distribution of food during funerals should be discouraged. These should never be done by the same persons.

4.9 Antimicrobial prophylaxis

Giving an antimicrobial to prevent transmission of Sd1 dysentery is *never* indicated. It has not been shown to be effective and it can hasten the emergence of resistant strains, making treatment of the disease more difficult.

5. PREPARATION FOR *SHIGELLA DYSENTERIAE* TYPE 1 EPIDEMICS

A strong national Control of Diarrhoeal Disease (CDD) Programme is the best long-term preparation for an epidemic of dysentery caused by Sd1. Countries with well-established national CDD programmes have effective disease surveillance systems, trained health professionals, reliably supplied health facilities, and ongoing health education activities. Programmes in various government ministries and departments work together to improve water supply, sanitation and food safety practices.

When an outbreak of dysentery occurs in the area or nearby, these activities need to be reinforced and applied to its control. If these activities are not yet established, they must be developed urgently. Specific preparatory measures are described below.

5.1 Coordinating committee

An interministerial committee should be developed to plan and coordinate the response to outbreaks of communicable diseases, including dysentery. The committee should include the manager of the national CDD programme. It may also be appropriate to establish similar committees at subnational levels. The committee's objective should be to ensure rapid implementation of effective control measures. Some specific functions of the committee should be to:

- make a comprehensive plan of preparation for an epidemic;
- coordinate the efforts of various governmental sectors;
- collaborate with regional and international organizations;
- collect and report information on dysentery cases and deaths;
- organize training;
- procure, store and distribute essential supplies;
- implement, supervise, monitor and evaluate control activities.

In the event that no such committee exists when an outbreak threatens, one should be created.

5.2 Surveillance and reporting

For surveillance and reporting purposes, the case-definition of dysentery is *diarrhoea with visible blood in the stool*. To detect outbreaks of dysentery, treatment facilities should record systematically and review regularly all cases of bloody diarrhoea. Records should include the name, age, date of visit and address for each patient, the clinical diagnosis, and the treatment given. Ideally, these data should be summarized and reported weekly to facilitate early detection of epidemics. A dysentery outbreak should be suspected whenever there is an unusual increase in the weekly number of patients with bloody diarrhoea or deaths from bloody diarrhoea.

When an outbreak of dysentery is detected, local and provincial (or national) health authorities should be notified immediately. Reports should specify the number of patients, their ages, the dates of onset of illness, and the names of towns or villages affected. Bacteriological studies should be done promptly to determine whether Sd1 is the cause (see Annex 7). The national CDD Programme manager or the Epidemic Control Unit of the Ministry of Health should be informed immediately of all bacteriologic results, so that appropriate control measures can be started. Reports of outbreaks should also be shared with neighbouring countries, as dysentery epidemics do not respect national borders. Although international notification of epidemic dysentery is not required, the local WHO representative and other appropriate authorities should be informed.

5.3 Laboratory

The overall role of the laboratory is described in section 8. Some steps that should be taken to prepare for a possible outbreak of Sd1 infection are as follows:

- *Prepare at least one laboratory for isolation of Shigella.* Not every laboratory needs to be capable of isolating *Shigella*. It is preferable to have one well-equipped laboratory with suitably trained staff to which specimens can be quickly and safely transported, than to have several that are equipped and staffed inadequately.

- *Obtain appropriate transport media.* See Annex 5.

- *Make provision for cold transport of stool specimens*. Specimens to be cultured for *Shigella* should be transported promptly to the laboratory at 4°C. See Annex 5.

- *Have the necessary supplies at the designated laboratory*. See Annex 6.

When an outbreak is reported the urgent need is to identify the causative organism and determine its antimicrobial susceptibility. Procedures for collection of stool specimens, identification of Sd1 and drug susceptibility testing are in Annexes 5, 7 and 8.

5.4 Treatment policy

The mainstay of treatment for Sd1 disease is appropriate antimicrobial therapy, which lessens the risk of serious complications and death. Other supportive measures used in treatment of acute diarrhoea should also be provided.

A national treatment policy for epidemic dysentery caused by Sd1 should be prepared that includes:

- giving an antimicrobial effective for Sd1;
- giving ORS solution or other fluids to prevent or treat dehydration;
- continued feeding;
- providing follow-up and referral for persons at increased risk of serious morbidity or death.

5.4.1 Selection of effective antimicrobials

The selection of recommended antimicrobials should be based on recent susceptibility testing of Sd1 strains from a nearby area or, after an epidemic develops, obtained locally. Guidelines for susceptibility testing are in Annex 8. Antimicrobials that should be considered are listed in Table 1. Antimicrobials should be selected that are:

- effective against at least 80% of local (or nearby) Sd1 strains;[4]
- given by mouth;
- affordable;
- available locally or rapidly obtainable.

Unfortunately, resistance of Sd1 to ampicillin and cotrimoxazole has become widespread. Nalidixic acid, formerly used as a "backup" drug to treat resistant shigellosis, is now the drug of choice in most areas, but resistance to it is also appearing. The fluoroquinolones and pivmecillinam (amdinocillin pivoxil) are still effective for most strains of Sd1, but most are costly and may not be readily available.

When the presence of Sd1 has not been confirmed, or its antimicrobial susceptibility is not yet known, nalidixic acid should be selected until more precise information is available.

Antimicrobials that are *not effective* for Sd1 are listed in Table 2. These include: (i) agents to which Sd1 strains are usually resistant, and (ii) those to which Sd1 strains are sensitive *in vitro*, but which penetrate poorly the intestinal mucosa where invasive Sd1 must be killed. These agents, and antimicrobials to

[4] If the best available antimicrobial has lower efficacy, e.g. 50%, it should be used until a more effective one can be obtained.

which Sd1 strains are resistant *in vitro*, should *not* be selected.

5.4.2 Use of antimicrobials when the supply is limited

When the supply of an effective antimicrobial is not sufficient to treat all cases, priority for treatment should be given to those at highest risk of death, as described in section 7.2. Vigorous efforts should also be made to obtain a sufficient stock of an effective antimicrobial for treatment of *all* cases of bloody diarrhoea. This may require importing an antimicrobial that is not available locally.

Table 1

Summary of antimicrobials for treatment of infections with *Shigella dy*

Agent	Resistance		Cost[a]	Availability	
	S. dysenteriae type 1	Other *Shigella*			
Ampicillin	Common	Variable	Medium	Wide	1 4
TMP-SMX	Common	Variable	Low	Wide	T S T
Nalidixic acid	Increasing	Infrequent	Medium	Moderate	1 4
Pivmecillinam	Infrequent	Rare	High	Limited	4 4
Ciprofloxacin	Rare	Rare	High	Limited	5 T
Norfloxacin	Rare	Rare	Medium	Limited	4 T
Enoxacin	Rare	Rare	High	Limited	2 T

[a] Low = <US$ 1.00; Medium = US$ 1-4.00; High = US$ 5-30.00. Cost will vary from place to place and over time. Costs prese treatment for an adult when antimicrobials are purchased in large quantities.

[b] All antimicrobials should be given for five days.

[c] To determine a child's dose, multiply the dose/kg by the child's weight. However, the child's dose should never be more than th

[d] New quinolones have not yet been approved for use in children below 12 years of age. There is growing evidence, however, tha effective. They are already used by some workers to treat children with serious illness caused by strains of Sd1 resistant to all otl

Table 2

Antimicrobials that are *not effective* against *Shigella dysenteriae* type 1

1. Antimicrobials to which strains of *Shigella* are usually resistant *in vitro*:

- Metronidazole
- Streptomycin
- Tetracyclines
- Chloramphenicol
- Sulfonamides

2. Antimicrobials to which *Shigella* may be sensitive *in vitro,* but with no documented efficacy *in vivo*:

- Nitrofurans (e.g. nitrofurantoin, furazolidone)
- Aminoglycosides (e.g. gentamicin, kanamycin)
- First- and second-generation cephalosporins (e.g. cephalexin, cefamandole)
- Amoxycillin

5.5 Emergency stocks of essential supplies

Health facilities must have access to adequate quantities of essential supplies, including: appropriate antimicrobials, oral rehydration salts (ORS) and intravenous fluids. During a dysentery epidemic these supplies may be needed quickly and in greater quantities than usual.

Sufficient stocks should be maintained at appropriate points in the drug delivery system. Small *reserve stocks* should be kept at local health facilities, larger *buffer stocks* at district or provincial sites, and an *emergency stock* at a central distribution point. Buffer stocks should be sufficient to meet a sudden increase in demand for specific supplies. Buffer and emergency stocks should be rotated regularly through the normal delivery system to avoid their becoming outdated.

A system is required to monitor the use of buffer and emergency stocks and to ensure their prompt replacement. The need for emergency supplies should be determined and individuals assigned to coordinate their procurement and distribution. The national coordinating committee should be responsible for procuring supplies and equipment from external agencies, ensuring that all drugs and materials are appropriate and avoiding duplication of requests (see section 5.1). A single central system for recording all incoming supplies and their distribution within the country is advised.

The supplies and equipment needed to manage 100 cases of dysentery are listed in Annex 10.

5.6 Training in case management

Medical and paramedical personnel should receive intensive and continuing training to ensure they are familiar with the most effective techniques for managing patients with acute diarrhoea, including dysentery. WHO can provide materials for clinical management training that emphasize hands-on practice in assessing and treating patients with diarrhoea.[5,6] These are appropriate for training health workers in dysentery case management as part of preparedness for epidemics.

5.7 Mobile control teams

Where peripheral health services are not prepared for epidemic dysentery, or are overwhelmed by it, mobile teams may be formed to:

- collect stool specimens for submission to a bacteriology laboratory;
- establish and operate temporary treatment centres;
- provide on-the-spot training in case management;
- supervise environmental sanitation and disinfection activities;
- carry out health education activities for the community;
- provide the emergency logistical support, such as delivery of essential supplies.

The teams may consist of doctors, nurses, paramedical staff, health educators and technicians. Briefing of team members on their duties, and any required training, should be done during preparation for a dysentery epidemic or when one is first detected.

6. PRINCIPAL STEPS IN THE MANAGEMENT OF PATIENTS WITH DYSENTERY CAUSED BY *SHIGELLA DYSENTERIAE* TYPE 1

Effective treatment of patients with bloody diarrhoea during an Sd1 epidemic consists of the following steps (see also Figure 1):

- Refer immediately to hospital persons who are severely malnourished, appear seriously ill or are in another high-risk category.

- Treat all cases promptly with an oral antimicrobial effective against local Sd1 strains.

- Treat and prevent dehydration with oral rehydration therapy, or intravenous (IV) therapy if severely dehydrated.

- Give frequent small meals of the patient's usual food; continue to breastfeed infants and young children.

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[5] *Diarrhoea management training course: guidelines for conducting clinical training courses at health centres and small hospitals*. Geneva, World Health Organization, 1990 (WHO document CDD/SER/90.2).

[6] *The management and prevention of diarrhoea; practical guidelines*, 3rd ed. Geneva, World Health Organization, 1993 (ISBN 92 4 154454 6).

7. DETAILS OF MANAGEMENT OF PATIENTS WITH DYSENTERY CAUSED BY *SHIGELLA DYSENTERIAE* TYPE 1

Public health messages should encourage all persons who develop bloody diarrhoea to report immediately to the nearest health facility with appropriately trained and supplied health workers. Community health workers on home visits should also help to find and refer cases for treatment. The treatment strategy is described below.

7.1 Diagnosing dysentery

The diagnosis of dysentery is made by observing blood in a fresh stool specimen or by asking the patient, or the mother of a child, whether the stools are bloody. These methods usually have equal sensitivity and precision. If there is doubt that a history of bloody stool is accurate, observation of a freshly passed stool is essential.

7.2 Identification of high-risk patients

Individuals at increased risk of death from dysentery caused by Sd1 are:

- children less than 5 years of age (infants, severely malnourished children[7] and children who have had measles in the past six weeks are at highest risk);
- adults 50 years of age or older;
- anyone who is dehydrated, has had a convulsion or is seriously ill when first seen;
- older children and adults who are obviously malnourished.

7.3 Referral to hospital

Children with severe malnutrition (weight-for-age less than 60% or weight-for-length less than 70% of National Center for Health Statistics medians) and any patient who is seriously ill should be referred immediately to hospital.

Other high-risk patients should also be referred to hospital, if space is available. Otherwise they should be treated as outpatients with careful follow-up to ensure they are improving in response to antimicrobial therapy.

7.4 Antimicrobial therapy

Appropriate treatment requires an oral antimicrobial that is effective against local strains of Sd1 (see Table 1). If possible, one should be selected that is effective against *all* Sd1 strains. If an effective antimicrobial is unavailable or in limited supply, treatment guidelines must be revised. Both situations are considered below.

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[7] This refers to children with weight-for-age <60%, or weight-for-length <70%, of National Center for Health Statistics median values, oedema of both legs, or mid-arm circumference less than 12.5cm (red band of a standard tape).

7.4.1 When an effective oral antimicrobial is available

Treat patients for five days. Give outpatients enough antimicrobial to last five days and instruct the patient (or mother) how to take it.

When an effective antimicrobial is taken, clinical improvement (i.e. feels better, fewer stools, less blood in the stool, less fever, less abdominal pain, improved appetite) normally occurs within 48 hours. Such improvement provides reassurance that the patient, although not yet fully recovered, is responding satisfactorily to treatment.

All high-risk patients treated as outpatients should be reviewed after two days of treatment. Any who are not improving, as defined above, should be admitted to hospital. Other outpatients who return after at least two days of treatment and have not improved should also be admitted to hospital.

If the antimicrobial these patients received was not effective against *all* local Sd1 strains *and* a second effective antimicrobial is available, the first one should be stopped and the second one given for five days. If a second antimicrobial for Sd1 is not available, the first one should be continued for five days. All patients should be given supportive care as described in section 7.5.

7.4.2 When an effective antimicrobial is in limited supply

Sometimes the supply of an effective antimicrobial is insufficient to treat all persons with dysentery. In such instances steps should be taken *urgently* to obtain a sufficient supply of an effective antimicrobial. Until this is achieved, the available supply of effective drug should be reserved for the high-risk patients described above and for patients whose illness worsens without antimicrobial therapy. *Antimicrobials that are known to be ineffective or to which the local Sd1 strain is resistant should not be given*. All patients, however, should receive the supportive treatment summarized below.

It should be emphasized that an effective antimicrobial should be given to *all* patients with dysentery caused by Sd1. Patients who do not receive an effective antimicrobial, even though not diagnosed as high-risk, may still have a severe or fatal outcome of their illness.

7.5 Supportive care

Optimal treatment of dysentery caused by Sd1 includes preventing or treating dehydration and continuing to feed, as described in WHO guidelines for the management of acute diarrhoea.[8]

7.5.1 Preventing and treating dehydration

Although dysentery is not usually associated with marked loss of fluid and electrolytes, the patient's state of hydration should be accurately assessed. If dehydration is detected, it should be treated at the health facility with ORS solution (for some dehydration) or IV fluids (for severe dehydration). Patients with dysentery and signs of dehydration are at increased risk for complications and should be re-evaluated after two days of treatment. All patients should be encouraged to take increased amounts of suitable fluids at home, such as ORS solution, rice water, soup, yoghurt-based drinks and plain water.

7.5.2 Nutritional management

Continued provision of nutritious food is important for all patients with dysentery. However, owing to

[8] *The treatment of diarrhoea: a manual for physicians and other senior health workers*. Geneva, World Health Organization, 1995 (WHO document WHO/CDR/95.3).

anorexia, patients may have to be coaxed to eat. Initially, food may be refused, but appetite usually improves after 1-2 days of effective antimicrobial therapy. Frequent small meals with familiar foods are usually better tolerated than a few large meals.

Infants and young children should breastfeed as often and as long as they want. Infants below 4 months who already take solid foods should continue to receive them. Mothers of infants younger than 4 months who are not exclusively breastfed should be advised and helped to establish exclusive breastfeeding. Infants older than 4 months, and young children, should be offered their usual foods. Young children convalescing from dysentery should be given an extra meal each day for at least two weeks to help them recover any weight lost during the illness. The caretakers of children with pre-existing malnutrition should be advised on appropriate feeding practices and the child monitored until substantial weight gain has been documented. See Annex 11 for a summary of feeding practices during and after diarrhoea.

Adults should eat easily-digestible, nutritious foods, avoiding those that are fried or spicy.

7.5.3 "Antidiarrhoeal" drugs

Drugs available for symptomatic relief of abdominal and rectal pain, or to reduce the frequency of stools (e.g. loperamide, diphenoxylate, paregoric) *should never be used* in the treatment of shigellosis as they may cause severe adverse effects.

7.6 Treatment of complications

7.6.1 Potassium depletion

Potassium depletion may be quite severe in shigellosis. It can be prevented by replacing faecal losses with ORS solution. Giving potassium-rich foods, such as bananas or green coconut water, is also helpful.

7.6.2 High fever

High fever (more than 39°C) can cause seizures in young children. It should be controlled by giving paracetamol. Reducing fever also improves appetite and reduces irritability.

7.6.3 Haemolytic-uraemic syndrome

Haemolytic-uraemic syndrome (HUS) is an unusual but serious complication of dysentery that affects the blood clotting system and kidneys. It may follow infection with Sd1 or *E. coli* O157:H7. The classic triad of symptoms is haemolytic anaemia, thrombocytopenia and renal failure. HUS may be mild, with rapid recovery, or severe, with kidney failure. Haemodialysis may be required. Clotting abnormalities can cause bleeding, and the red blood cell count may be low. Transfusions of whole blood or platelets are often needed in severe cases. With adequate treatment, many HUS patients recover fully.

HUS should be suspected when a dysentery patient develops easy bruising and has little or no urine output. The diagnosis of HUS can be made by the following: (i) a low haematocrit, (ii) a blood smear showing fragmented red blood cells, (iii) a low platelet count, or platelets not seen on the blood smear, and (iv) elevated levels of blood urea nitrogen or serum creatinine. When this occurs, stop giving potassium-rich foods or fluids, including ORS solution, and refer the patient to hospital.

8. Role of the laboratory

The principal tasks of the laboratory are to:

- isolate Sd1 when an epidemic is first reported;
- determine the antimicrobial susceptibility of the Sd1 isolates to guide antimicrobial therapy;
- monitor antimicrobial susceptibility of Sd1 isolates regularly during the epidemic to detect any important changes.

After an epidemic caused by Sd1 has been confirmed, it is not necessary to examine specimens from all cases or contacts. In fact, this should be discouraged as it places an unnecessary burden on laboratory facilities and is not required for effective treatment.

The laboratory must inform government health officials, clinicians and epidemiologists promptly of all relevant findings. National laboratories may obtain technical assistance from WHO or one of its Collaborating Centres (Annex 12).

8.1 Determine the cause of the epidemic

When a dysentery epidemic is first reported, stool specimens should be collected from 10-20 untreated cases to determine if Sd1 is the cause. The method for collecting and transporting stools is described in Annex 5, and the method for isolating and identifying Sd1 is described in Annex 7. A list of essential supplies is found in Annex 6. If Sd1 is not identified, stool specimens should be cultured for *E. coli* O157:H7 (see Annex 9).

If difficulty is encountered, WHO reference laboratories can assist national laboratories to isolate and identify *Shigella*, including Sd1, from faecal samples. Specimens should be shipped by express air mail (see Annexes 5 and 12).

8.2 Determine antimicrobial susceptibility of *Shigella dysenteriae* type 1

The technique for determining antimicrobial susceptibility of Sd1 is described in Annex 8. It is recommended that initial antimicrobial susceptibilities be confirmed at a WHO reference laboratory.

The susceptibility of Sd1 to antimicrobials can change dramatically during an epidemic. It is important, therefore, that susceptibility be reassessed regularly, for example, every 2-6 months. For seasonal epidemics, susceptibility testing should also be performed at the end of the epidemic season to allow antibiotic policy for the following season to be determined. A plan should be established as part of epidemic preparedness to collect 10-20 specimens from untreated patients in different affected areas and transport them to the designated laboratory. The antimicrobial susceptibility of Sd1 isolates should be determined and any important changes reported promptly, so that necessary changes in recommended antimicrobial treatment can be made.

8.3 Reference laboratories

A national reference laboratory should be able to isolate and identify *Shigella*, including Sd1, and perform antimicrobial susceptibility testing, or at least have access to an international reference laboratory with those capabilities. The reference laboratory should also be responsible for training regional and local health staff in appropriate isolation and transport techniques, monitoring the quality of the laboratory services, and ensuring that the results of laboratory testing are disseminated to regional

and local health staff.

9. AFTER AN OUTBREAK

Careful clinical surveillance should be continued to ensure that sporadic cases of shigellosis are promptly detected and treated. Efforts to improve personal and domestic hygiene, water supplies and sanitation to help prevent a recurrence of the epidemic should also be continued.

Routine laboratory examinations of food and water are not likely to be helpful. The experience gained during the epidemic should be used to strengthen the capacity of the national CDD programme to control all endemic acute diarrhoea, including shigellosis, and help prevent further epidemics.

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ANNEX 1

Sample public health messages for prevention of *Shigella dysenteriae* infection

The following sample messages are valid for all forms of acute diarrhoea, including dysentery and cholera. They should be adapted to local conditions and translated into local languages:

THREE SIMPLE RULES FOR PREVENTING DYSENTERY

1. Cook your food
2. Boil or chlorinate your drinking-water
3. Wash your hands

ARE YOU PROTECTED FROM DYSENTERY?
DO YOU PREPARE FOOD SAFELY?

Cooking kills dysentery germs

- Thoroughly cook all meats, fish and vegetables.
- Eat them while they are hot.

Washing protects from dysentery

- Wash your *hands* before preparing or serving food.
- Wash your *dishes and utensils* with soap and water.
- Wash your *cutting board* especially well with soap and water.

Peeling protects from dysentery

- Eat only fruits that have been freshly peeled, such as oranges and bananas.

KEEP IT CLEAN: COOK IT, PEEL IT, OR LEAVE IT!

ARE YOU PROTECTED FROM DYSENTERY?
IS YOUR DRINKING-WATER BOILED OR TREATED?

Even if it looks clean, water can contain dysentery germs.

Water for drinking can be made safe in two ways:

- *Boil* it to kill dysentery germs.
- *Chlorine* kills dysentery germs: use three drops of *chlorine solution* for each litre of water, mix well, and leave it for half an hour before drinking.

To make the chlorine solution: mix three level tablespoons (33 grams) of bleaching powder in one litre of water.[a]

DRINK ONLY SAFE WATER

[a] This quantity is for a bleaching powder that contains 30% concentration by weight of available chlorine. The quantity to be recommended must be adapted for the bleach available on the local market.

ARE YOU PROTECTED FROM DYSENTERY?
IS YOUR DRINKING-WATER STORED SAFELY?

Clean water can become contaminated again if it is not stored safely.

Store drinking-water in a clean container with a small opening or a cover. Use it within 24 hours.

Pour from the water container - do not dip a cup into the container.

KEEP IT CLEAN: STORE DRINKING-WATER SAFELY

ARE YOU PROTECTED FROM DYSENTERY?
DO YOU WASH YOUR HANDS?

The germs that cause dysentery are invisible. They can be carried on your hands without your knowing it.

Always wash your hands:

- after you use the toilet or latrine, or clean up your children;
- before you prepare or serve food;
- before you eat or feed your children.

What is the best way to wash your hands?

- Always use soap or ash.
- Use plenty of clean water.
- Wash all parts of your hands - front, back, between the fingers, under the nails.

KEEP IT CLEAN: WASH YOUR HANDS

ARE YOU PROTECTED FROM DYSENTERY?
DO YOU USE A TOILET OR LATRINE?

Dysentery germs live in faeces. Even a person who is healthy might have dysentery germs in the faeces.

- *Always use* a toilet or latrine. If you don't have one - build one.
- Keep the toilet or latrine *clean*.
- Dispose of *babies' faeces* in the toilet or latrine (or bury them).
- *Wash your hands* with soap (or ash) and clean water after using the toilet or latrine.

KEEP IT CLEAN: USE A TOILET OR LATRINE

ANNEX 2

Rules for preparing food safely to prevent dysentery[9]

1. Cook foods thoroughly

Foods can easily become contaminated with the germs that causes dysentery. Thorough cooking will kill the germs, but remember that *all parts of the food* must become hot. Do not eat uncooked foods, unless you peel or shell them yourself.

2. Eat cooked foods immediately

When cooked foods cool to room temperature, bacteria can begin to grow. To be on the safe side, eat cooked foods as soon as they come off the heat. When there is a delay between cooking and eating food, as when food is sold in restaurants or by street vendors, it should be kept over heat, at 60°C or more, until it is served.

3. Store cooked foods carefully

If foods must be prepared in advance or kept as leftovers, be sure to store them in a refrigerator or ice box below 10°C or in a hot box kept continuously above 60°C. Otherwise, cooked foods that have been stored for more than two hours must be thoroughly reheated before being eaten.

4. Reheat cooked foods thoroughly

Reheating foods thoroughly before eating is your best protection against germs that may have grown during storage. Thorough reheating means that *all parts of the food* must become hot. Eat food while it is still hot.

5. Avoid contact between raw foods and cooked foods

Safely cooked food can become contaminated, and potentially dangerous, through even the slightest contact with raw food. Contact can be direct, as when raw fish comes into contact with cooked foods. It can also be indirect, as when a cutting surface and knife are used to prepare raw fish and then, without washing, to slice cooked food. Doing so can reintroduce all the potential risks of illness that were present before cooking.

6. Wash hands frequently

Wash hands thoroughly before you start preparing food and after every interruption, especially if you have to "change" or clean up the baby or have used the toilet or latrine.

7. Keep all kitchen surfaces clean

Any surface used to prepare food must be kept absolutely clean. Think of every food scrap, crumb or spot as a potential source of germs. Cloths used for washing or drying food preparation surfaces, dishes and utensils should be changed every day and boiled before reuse.

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[9] Adapted from Annex 6, Golden rules for safe food preparation, in *Health surveillance and management procedures for food-handling personnel:* report of a WHO consultation. Geneva, World Health Organization, 1989 (WHO Technical Report Series, No.785).

8. Use safe water

Safe water should be used when preparing food that will not be cooked or when making ice for drinks. If there are any doubts about the water, boil it or treat it with chlorine before it is used.

ANNEX 3

Making water safe by chlorination

The following guidelines should be translated into messages that take into account locally-available products and measuring devices, and whether the instructions are for home or institutional use.

Make a stock solution of chlorine (1% concentration by weight of available chlorine). Add to 1 litre of water:

Product (% concentration by weight of available chlorine)	*Amount*	
Calcium hypochlorite (70%)	15	grams
Or		
Bleaching powder or chlorinated lime (30%)	33	grams
or		
Sodium hypochlorite (5%)	250	ml
Or		
Sodium hypochlorite (10%)	110	ml

If products with these concentrations of chlorine are not available, adjust the amount used according to the available concentrations.

Store the stock solution in a cool place in a closed container that does not admit light. The stock solution must be used no later than one month after it is made.

Use the stock solution to make water safe. Add water to stock solution to ensure complete mixing:

Water.....to add to.....Stock solution	
1 litre	0.6 ml or 3 drops
10 litres	6 ml or 30 drops
100 litres	60 ml or 300 drops

Allow the chlorinated water to stand for at least 30 minutes before using it. The residual chlorine level after 30 minutes should be between 0.2 and 0.5 mg/litre.

If the water is turbid (not clear, with a lot of suspended solid matter):

- filter it before chlorination,

  or

- boil it vigorously for 1 minute, instead of treating it by chlorination.

Recommended chlorine levels in water distribution systems in areas affected by epidemic dysentery

The minimum levels of free residual chlorine necessary for safe water are:

- at all sampling points in a piped water system..... 0.5 mg/litre
- at standposts ..... 1.0 mg/litre
- in tanker trucks, at filling ..... 2.0 mg/litre

Regular monitoring is required to ensure that these minimum levels of chlorine are maintained.

ANNEX 4

Building a ventilated improved pit latrine[10]

A *ventilated improved pit latrine* is a practical means of disposing of human excreta and may be a good solution for use in rural areas. The decision on the type of latrine to be selected should take account of local factors such as type of soil and density of population.

The latrine must be constructed at least 30 metres from wells or other sources of drinking-water and, where possible, at least six metres from houses. It should not be located uphill from the water source or dug in marshy soil.

A latrine 2 metres deep with an opening 1 metre x 1 metre can be used by a family of five for 2-4 years. (This assumes an accumulation rate of between 60 and 100 litres per person per year.) To keep bad odours and flies to a minimum, ventilation can be provided by an external vertical vent, topped by a fly screen. The edges of the pit should be raised above ground level to prevent rain or other water from draining into it. The latrine should have a concrete or wooden slab that reaches the walls of the superstructure. Where possible, concrete reinforced with steel wires at least 8 mm in diameter and 150 mm apart should be used because of its durability and resistance.

The slabs and floor should be washed daily and disinfected regularly with cresol or bleaching powder. After the pit is loaded to two-thirds of its capacity (1.3 metres height), it should be filled with soil and compacted, and a new pit should be dug.

[10] For more specific instructions, see Cairncross S. *Small scale sanitation.* London, London School of Hygiene and Tropical Medicine, 1988.

ANNEX 5

Collection and transport of stool specimens for *Shigella*

Specimens that cannot be cultured within one hour of collection should be placed in a transport medium and refrigerated immediately. Unlike some organisms, *Shigella* will die, even in transport media, if they are not refrigerated.

Selection of transport media

Cary-Blair transport medium is a semi-solid medium useful for the preservation and transport of specimens for *Shigella*, as well as *Escherichia coli*, *Salmonella*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Yersinia enterocolitica*. It is stable when stored in tightly sealed containers. It can be used for 18 months or longer under proper conditions of storage, provided there is no loss of volume and no evidence of contamination or colour change. Other transport media that are similar to Cary-Blair are Amies' and Stuart's transport media.

Buffered glycerol saline (BGS) is also useful for transporting specimens for *Shigella* as well as *Escherichia coli* and *Salmonella*. BGS is considered to be better than Cary-Blair for *Shigella*, provided that the BGS is still alkaline, as indicated by the pink colour that persists after the addition of faeces. BGS is unsuitable for *Vibrio* or *Campylobacter*. Its disadvantages are that it is a liquid medium and that it can be used for only one month after it is made.

Selection of cases for bacteriologic sampling

When an outbreak of dysentery occurs, laboratory analysis of a small number of adequately collected clinical specimens is sufficient to provide the diagnosis. The key is to collect the specimens properly and to transport them rapidly to a fully equipped clinical laboratory. This approach permits rapid diagnosis of outbreaks at low cost. 10-20 cases should be selected for sampling at each investigation site. Cases should meet all of the following criteria:

- onset of illness less than four days before sampling;
- currently having bloody diarrhoea;
- have not received antibiotic treatment for this illness;
- consent to give a specimen.

Collection of specimens

Two swabs of rectal contents or of fresh stool (less than one hour old) should be collected from each case selected. If possible, the Cary-Blair transport media should be refrigerated for an hour before use so the swabs can be placed into cool medium. Insert swab into the Cary-Blair first in order to moisten it, then insert it 1-1½ inches into the rectum and rotate it gently. Remove the swab and examine it to ensure that the cotton tip is stained with faeces. Insert the swab immediately into the tube of transport media. Push the swab to the bottom of the tube of medium. Repeat with the second swab, placing it in the same tube as the first swab. Break off the top part of the sticks. Tighten the screw top firmly.

Labelling of specimens

Use the attached Stool Specimen Data Sheet to record information on each case. Assign numbers to collected specimens in consecutive order. Always write the numbers on the frosted portion of the specimen tube using an indelible marker pen. If no frosted area is present, apply firmly a piece of first aid tape and write on it.

Transport of specimens

Refrigeration of specimens after collection is essential. If the specimens will arrive at the laboratory within two days, they can be refrigerated at 4°C. Pathogens can still be recovered from refrigerated samples up to seven days after collection, although the yield decreases after the first two days. Refrigeration during transport can be achieved for up to 36 hours by shipping in a well insulated box with frozen refrigerant packs or wet ice.

If it is impossible for specimens to reach a laboratory within two days, they can be frozen, although this will decrease the number of organisms present and the likelihood of isolating the pathogen. They should be frozen as soon as possible after collection and held at -20°C. Freezing at conventional freezer temperatures (-5° to 0°C) is not acceptable, as it allows thawing and refreezing, which will quickly reduce the number of organisms present. Frozen specimens should be shipped with dry ice, observing the following precautions:

- protect the specimens from direct contact with dry ice, as intense cold can crack the glass tubes;
- protect the specimens from carbon dioxide by sealing the screwcaps with electrical tape or by sealing the tubes in a plastic bag;
- ensure that the container is at least one-third full of dry ice. If specimens are shipped by air and more than 2 kg of dry ice is used, special arrangements may be necessary with the air carrier.

The shipping arrangements should be determined before specimens are collected. Within-country shipping may be by ground or by air. To ship longer distances (e.g. to a reference laboratory or WHO Collaborating Centre) overnight express air mail is ideal. As wet ice in the box will not last more than 36 hours, arrangements should be made for immediate pickup at the receiving airport. When shipped, communicate the following information immediately to the receiving laboratory: the air bill number, the flight number, and the times and dates of departure and arrival of the flight. Address the package clearly, including the name and telephone number of the receiving laboratory. Write in large letters: EMERGENCY MEDICAL SPECIMENS; CALL ADDRESSEE ON ARRIVAL; HOLD REFRIGERATED.

Contents of transport kit

- Reinforced cooler suitable for shipping
- 120 tubes of Cary-Blair transport medium
- 250 sterile rectal swabs
- 1-3 freezable cool-packs
- 1 indelible laboratory marker
- 1 roll of first aid tape (for labelling tubes)
- 12 patient registry forms
- Instructions

Cary-Blair medium

Sodium thioglycolate	1.5 g
Disodium hydrogen phosphate (Na_2HPO_4)	1.1 g
Sodium chloride	5.0 g
Agar	5.0 g
Distilled water	991.0 ml

Preparation: Dissolve the ingredients in the water while heating in a boiling water-bath, stirring until the solution is clear (do not allow it to boil). After cooling to 50°C, add 9 ml of freshly prepared 1% calcium chloride solution and adjust the pH to 8.4 (with N/10 sodium hydroxide). Dispense 7 ml amounts into cleaned and sterilized 9 ml screw-capped bottles (e.g. Bijou bottles), leaving a small air space at the top and the caps loosened. Sterilize by steaming at 100°C or in a boiling water-bath for 15 minutes, and tighten the caps after sterilization. Record the batch date on the label and store in a cool dark place.

STOOL SPECIMEN DATA SHEET
BACTERIOLOGIC SURVEILLANCE

Country _____

Province _____

Area/Zone _____

Village/Town _____

No.	Date of Collection	Name	Age (yr)/ Sex (M:F)	Bloody stool Yes/no	Taken antibiotics Yes/no*
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

* If antibiotics were taken, list type of antibiotic, dose and number of days taken

Collected by: Name _____

Title _____

Transmit results to: Name _____

Address _____

____

Phone/Fax/Telex ________________________________

ANNEX 6

Supplies needed for laboratory identification of *Shigella dysenteriae* (for 100 cases)

1.	Rectal swabs	100
2.	XLD medium	3 x 100 g
3.	MacConkey agar	3 x 100 g
4.	Kligler agar	2 x 100 g
5.	Mueller Hinton agar	2 x 100 g
6.	Diagnostic antisera:	Polyvalent: *S. dysenteriae* (group A) *S. flexneri* (group B) *S. boydii* (group C) *S. sonnei* (group D) Monovalent: *S. dysenteriae* type 1
7.	Disposable Petri dishes (9 cm)	200
8.	Test tubes (13 x 100 mm)	200
9.	Disposable Bijou bottles	200
10.	Antibiotic discs for susceptibility tests (50 of each):	Ampicillin TMP/ SMX Nalidixic acid Pivmecillinam Ciprofloxacin (or other fluoroquinolone)
11.	Control strains (susceptible and resistant)	

ANNEX 7

Laboratory identification of *Shigella*[11]

Enrichment

No enrichment medium is suitable for *Shigella.*

Preparation of faecal suspension

Suspend faeces from rectal or faecal swab in a tube containing 1 ml of saline (0.85% NaCl). Wash the swab thoroughly in the saline by swirling the tube, and rotate the swab against the side of the tube to express the fluid from the swab.

Portions of formed stools should also be suspended in saline to make a turbid suspension. Liquid stools require no addition of saline.

The plate can be inoculated with the faecal suspension, or directly from the swab, and then streaked with a loop.

Direct inoculation of agar plates

Use a moderate inoculum (2 or 3 loopfuls of faecal suspension). Incubate plates at 35-37°C for 18-24 hours.

Inoculate a general purpose plating medium of low selectivity and one of moderate or high selectivity. MacConkey agar is recommended as a medium of low selectivity. MacConkey agar with 1 mcg/ml of potassium tellurite has been reported to be particularly useful for *S. dysenteriae* type 1 (Sd1). Use a small inoculum. Incubate at 35-37°C for 18-24 hours.

Xylose-lysine-desoxycholate (XLD) agar is recommended as a medium of moderate or high selectivity for isolation of *Shigella*. Desoxycholate citrate agar (DCA) is a suitable alternative.

Do not use salmonella-shigella (SS) agar, as it often inhibits growth of Sd1.

Each new batch of medium should be controlled for quality before routine use by inoculating it with known reference strains and observing their growth and colony characteristics.

Identification of colonies on plating media

Colonies suspicious for *Shigella* will appear as follows:

MacConkey agar: Convex, colourless, 2-3 mm

[11] For additional information see: *Manual for laboratory investigations of acute enteric infections.* Geneva, World Health Organization, 1987 (WHO document CDD/83.3 Rev. 1).

XLD agar: Red, smooth, 1-2 mm
DCA agar: Colourless, translucent, 2-3 mm

Identify well separated colonies of typical appearance to be transferred from each of the plating media for further testing by making a mark on the bottom of the Petri plate.

Whenever possible a person experienced with the identification of *Shigella* should train laboratory workers who are not familiar with its identification.

Inoculation of Kligler iron agar (KIA)

Pick three characteristic colonies from the plating media and inoculate into KIA as follows: stab the butt and then streak the slant with a zig-zag configuration. Pay attention to proper labelling of the tubes. If screw-cap KIA tubes are used, make sure that the caps are loose. Incubate overnight. On the following morning, examine the reactions in the KIA tubes. Tubes suspicious for *Shigella* will have an acid (yellow) butt and an alkaline (red) slant. They will not produce gas (no bubbles or cracks in the agar) and will not produce hydrogen sulfide (no black along the stab line).

Triple sugar iron agar (TSI) can also be used for the identification of *Shigella.* It will give the same reactions as KIA.

Serological tests of cultures suspected of being *Shigella*

Agglutination tests are carried out on a clean glass slide. Use a straight wire to remove a portion of the growth from the surface of the KIA slant and emulsify in a 3 mm loopful of physiological saline. Mix thoroughly by tilting back and forth for about 30 seconds and then examine carefully to ensure that the suspension is smooth and does not show clumping due to auto-agglutination. If clumping occurs, the culture is rough and cannot be serotyped. If the suspension is smooth (turbid and free-flowing), add one loopful of antiserum, mix well using the loop, and observe for agglutination over a period of 60 seconds against a dark background. If the reaction is positive, clumping will appear within 30 seconds to one minute. Interpret the agglutination tests as shown below:

If agglutination occurs with *group A*, report:	*Shigella dysenteriae*
Test with S. dysenteriae type 1 antiserum. If positive, report:	*S. dysenteriae* type 1
If agglutination occurs with *group B*, report:	*Shigella flexneri*
If agglutination occurs with *group C*, report:	*Shigella boydii*
If agglutination occurs with *group D*, report:	*Shigella sonnei*

For further details on identifying *Shigella* and other *Enterobacteriaceae*, refer to the WHO *Manual for laboratory investigations of acute enteric infections*, CDD/83.3 Rev.1 (1987).

Media preparation

MacConkey agar, desoxycholate agar, and Kligler's iron agar are commercially available as pre-mixed powders. Preparation of media from individual ingredients is described below.

1. MacConkey agar (modified)

Peptone	20.0	g
Lactose	10.0	g
Bile salts	1.5	g
Sodium chloride	5.0	g
Agar	14.0	g
Neutral red	0.03	g
Crystal violet	0.001	g
Distilled water to give final volume of	1.0	litre

It may also be prepared with meat extract broth as indicated below:

Meat extract broth	1.0	litre
Peptone	10.0	g
Lactose	10.0	g
NaCl	5.0	g
Bile salts	1.5	g
Agar (certified bacteriological grade)	14.0	g
Add solutions 1 and 2, as described below		

Preparation of base medium: Add progressively and dissolve lactose, bile salts, peptone, and NaCl in one litre of meat extract broth while heating at approximately 80°C in a water-bath and stirring. Dissolve the agar by heating the broth in a boiling water-bath. Adjust the pH to 7.2-7.4 with 0.1 N sodium hydroxide. Distribute in 200-ml amounts in screw-capped bottles with caps loosened; sterilize by autoclaving at 121°C for 15 minutes. Tighten the caps after sterilization. Record the batch date on the label and store.

Preparation of Solution 1: Dissolve 1 g of neutral red in distilled water; make up to a volume of 100 ml. Heat the solution in steam at 100°C for 30 minutes. Label and store in a cool area or at 4°C.

Preparation of Solution 2: Dissolve 0.1 g of crystal violet in distilled water; make up to a volume of 100 ml. Heat the solution in steam at 100°C for 30 minutes. Label and store in a cool area or at 4°C.

Pouring plates: Melt 200 ml of the base solution in a boiling water-bath. Cool to about 60°C. Add aseptically 0.6 ml of solution 1 and 0.4 ml of solution 2. Mix well and pour into 90 mm sterile Petri dishes.

Sterility test: Incubate the plates at 37°C for 24 hours and examine for contamination.

Performance test: Prepare an 18-hour broth culture from the stock cultures of *S. typhi*, *E. coli*, and *S. flexneri*.

- Mix the broth cultures of *S. typhi* and *E. coli* in a 1:10 ratio (volume to volume). Prepare a 10^6 dilution of the mixture (take one 4 mm loopful of the mixture and add it to 10 ml of sterile physiological saline, mix well, then take a 4 mm loopful of the dilution and add to another 10 ml of sterile saline).

- Prepare a 10^6 dilution of the *S. flexneri* broth culture in a similar manner.

- Inoculate the plating media with 4-mm loopfuls of the dilute mixtures of *S. typhi*, *E. coli* and *S. flexneri*.

- Incubate the plates at 37°C overnight and examine for growth of typical colonies.

2. Xylose lysine desoxycholate agar (XLD agar)

Basal medium:

Xylose	3.75	g
L-lysine HC1	5.0	g
Lactose	7.5	g
Sucrose	7.5	g
Sodium chloride	5.0	g
Yeast extract	3.0	g
Phenol red (0.2% solution	40.0	ml
Agar	15.0	g
Distilled water	960.0	ml

Directions for complete medium: Add all ingredients to the water and heat to boiling to obtain a complete solution. Cool to 50-55°C, adjust reaction so that pH after sterilization will be 6.9, and autoclave at 121°C for 15 minutes. Cool to 50-55°C and add aseptically the following solutions in the amount indicated:

- 20 ml of thiosulfate-citrate solution (to prepare, dissolve 34 g of sodium thiosulfate and 4 g of ferric ammonium citrate in 100 ml of water; sterilize by filtration);

- 25 ml of a 10% aqueous solution of sodium desoxycholate (sterilize by filtration or by autoclaving at 121°C for 15 minutes).

Mix well, readjust pH if necessary to 6.9, and pour in 15-20 ml amounts into Petri dishes.

3. Desoxycholate citrate agar (DCA) (modified)

Base medium:

Meat extract broth	1.0	litre
Proteose peptone	10.0	g
Lactose	10.0	g
Neutral red 1% solution	2.5	ml
Agar	17.0	g

Solution 1:

Sodium citrate	17.0	g
Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)	17.0	g
Ferric ammonium citrate	2.0	g
Distilled water	100.0	ml

Solution 2:

Sodium desoxycholate	10.0	g
Distilled water	100.0	ml

Preparation of base medium: Adjust the reaction of the broth to pH 8.0-8.4 and dissolve the agar by heating in a boiling water-bath or in steam at 100°C. Filter the molten agar immediately upon removal from heating through multilayer surgical gauze. Adjust to pH 7.4; add 2.5 ml of freshly prepared 1% solution of neutral red and 10 g of lactose and 10 g of proteose peptone. Mix well and distribute in 200-ml amounts in screw-capped bottles. Sterilize by heating in steam at 100°C for one hour followed by autoclaving at 110°C for 10 minutes. Tighten the caps, record batch date on the label and store at 4°C.

Preparation of plates: Melt 200 ml of the base medium and cool to about 80°C. Add aseptically 10 ml of solution 1 and the appropriate volume of solution 2 (as indicated in the following procedure on titration) using two different pipettes; mix well after each dilution. Distribute into sterile Petri dishes. The medium must cool rapidly, otherwise it may become too soft. Record the batch number and date on the label.

Titration of sodium desoxycholate: Melt seven bottles of the base medium and label from 6 to 12. Add 10 ml of solution 1 to each bottle. Add respectively 6, 7, 8, 9, 10, 11, and 12 ml of solution 2 to the bottles 6, 7, 8, 9, 10, 11, and 12. Mix well and pour plates (label plates with the same numbers as the bottles). Select the plates that give the best growth of *Salmonella* and *Shigella*. Record the volume of solution 2 used.

Use: This medium is selective for *Salmonella* and *Shigella*. *Salmonella* will produce raised colourless or translucent colonies. *Shigella* will produce opaque ground-glass colonies. It must be remembered that other non-lactose-fermenting organisms will grow on DCA, and these need to be differentiated from *Salmonella* and *Shigella* by biochemical tests. Lactose-fermenting organisms form raised colonies often surrounded by a red halo.

4. Kligler's iron agar (KIA)

Meat infusion broth	1.0 litre
Peptone	5.0 g
Proteose peptone	5.0 g
NaCl	5.0 g
Lactose	10.0 g
Glucose	1.0 g
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)	0.2 g
Sodium thiosulfate	0.3 g
($Na_2S_2O_3 \cdot 5H_2O$)	0.2 g
Phenol red solution 0.5%	6.0 ml
Agar	12.0 g

Preparation: Dissolve the agar in the meat infusion broth (see below), or alternatively in meat extract broth, by heating in a boiling water-bath or in steam at 100°C. Bring the molten nutrient agar to 80°C in a water-bath. Add and dissolve the lactose, peptone, proteose peptone, NaCl, glucose, ferrous sulfate, and sodium thiosulfate and mix well. Adjust the pH to 7.4. Add 6 ml of 0.5% solution of phenol red and mix well. Distribute in screw-cap tubes (15 x 150 or 16 x 160 mm) in 5-6 ml amounts and sterilize by autoclaving at 121°C for 15 minutes. Allow the medium to cool and set with a slant of 2.5 cm and a butt 2.5 cm deep. Record batch number and date on the label and store at room temperature not exceeding 25°C.

For preparation of meat infusion broth and meat extract broth, see below. Alternatively, an additional 10 g of peptone, 3 g of beef extract, 3 g of yeast extract, and one litre of distilled water may be used in place of meat infusion broth.

Sterility test: Incubate the tubes at 37°C for 18-24 hours and examine for contamination.

Performance test: Inoculate five tubes with the following cultures: *S. typhi*, *S. paratyphi* B, *E. coli*, *Citrobacter freundii* and *Shigella sonnei*. Incubate at 37°C for 18-24 hours and examine for correct reactions.

5. Preparation of meat extract broth

Put 500 g of lean minced meat (beef heart or fat-free meat) into a pan or casserole and add one litre of water; place it in the refrigerator (4°C) overnight. Next morning, bring to a boil and simmer for 15 minutes, while stirring with a glass rod. Filter through a wet paper filter to remove fat. Add water to make one litre (to replace that lost in boiling).

6. Preparation of meat infusion

Several media contain an infusion from, for example, beef heart or veal heart. Heat one litre of 1/20 N aqueous sodium hydroxide to boiling and add 1000 g of minced fat-free fresh meat or organ. Mix thoroughly, bring to a boil, and simmer for 20 minutes stirring frequently. The reaction of the mixture should be about pH 7.5. Strain through several layers of cheesecloth, squeeze out excess liquid, adjust the volume to 1000 ml with distilled water, and use the liquid immediately for making up media as specified in the formulations.

Add 450 ml of the infusion to 550 ml of distilled water, then add other ingredients. Dispense in 5 ml volumes in tubes, and sterilize by autoclaving at 121°C for 15 minutes. Adjust final pH to 7.4.

ANNEX 8

Antimicrobial susceptibility testing of *Shigella*[12]

Types of susceptibility tests

The dilution test: For quantitative estimates of antimicrobial activity, dilutions of the antimicrobial may be incorporated into broth or agar medium, which is then inoculated with the test organism. The lowest concentration that prevents visible growth after overnight incubation is known as the minimum inhibitory concentration (MIC) of the agent.

The diffusion test: Paper discs or tablets impregnated with the antimicrobial are placed on agar medium uniformly seeded with the test organism. A concentration gradient of the antimicrobial is formed by diffusion from the disc and the growth of the test organism is inhibited at a distance from the disc that is related, among other factors, to the susceptibility of the organism.

There is an approximate linear relation between the log MIC as measured by a dilution test and the inhibition zone diameter obtained in the diffusion test. A regression line expressing this relation can be obtained by testing a large number of strains by the two methods in parallel, and should be established when locally prepared discs are used.

The interpretation of the diffusion test results should always be based on this correlation between MIC and inhibition zone, combined with information on the therapeutically obtainable concentrations of the antimicrobials.

The procedure for the *diffusion test* is described below.

Media

Agar: Use one of the media recommended by the manufacturer of the discs. This will allow you to interpret inhibition zones using guidelines recommended by the manufacturer.

If the recommended media are not easily obtainable, one of the commercially available media (e.g. Mueller-Hinton medium, DST agar, Sensitest agar, Iso-sensitest agar, Wellcotest, Sulphonamide-Antagonist-Free medium) may be tested to see whether there are major differences in the inhibition zones as compared with the recommended media.

If a new medium is to be used, it should be checked to determine:

- whether the inhibition zone of the control strains (see table) is correct;
- whether the pH is correct;
- if possible, whether the concentration of divalent ions is correct.

The medium should be poured in Petri dishes with an agar depth of 4 mm or more (25 ml in a

[12] For additional information see: *Manual for laboratory investigations of acute enteric infections.* Geneva, World Health Organization, 1987 (WHO document CDD/83.3 Rev. 1).

9 cm plate). Dry the plates before use. Store unused plates for not more than two weeks in the refrigerator, preferably in a sealed plastic bag. Take care that they do not become too dry.

Table: Quality control - susceptibility of control strains

Antimicrobial	Disc potency	Zone diameter of inhibition (mm)	
		S. aureus (ATCC 25923)	*E. coli* (ATCC 25922)
Ampicillin	10 µg	24-35	15-20
Fluoroquinolone (Ciprofloxacin)	5 µg	22-30	30-40
Nalidixic acid	30 µg	---	21-25
Pivmecillinam	10 µg	---	23-29
Sulfamethoxazole-Trimethoprim	25 µg	24-32	24-32

Nutrient broth: Any nutrient broth that is available in the laboratory can be used, e.g. trypticase soy broth (for preparation, see below.) The broth should be distributed in 3-5 ml quantities and sterilized by autoclaving.

Trypticase soy broth:

Pancreatic digest of casein	17.0 g
Papaic digest of soy meal	3.0 g
Sodium chloride	5.0 g
Dipotassium phosphate	2.5 g
Dextrose	2.5 g
Distilled water	1.0 litre

Final pH 7.3

To prepare, dissolve the ingredients in the water, dispense, and autoclave at 121°C for 15 minutes.

Inoculum for the indirect susceptibility test

The indirect susceptibility test should be used for faecal bacteria. This requires that *Shigella* are inoculated from a pure culture. For most disc methods semiconfluent growth is recommended, and it is essential to use a standardized inoculum in order to perform a reliable susceptibility test. Use the inoculation method recommended by the manufacturer of the discs or tablets you are using. One of the following is usually recommended:

Kirby-Bauer inoculum:

- Make a turbidity standard by adding 0.5 ml of 1.175% (w/v) barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) solution to 99.5 ml of 1% sulphuric acid. The turbidity standard solution should be placed in a tube identical to the one used for the broth sample. It can be stored in the dark at room temperature (22-25°C) for six months, provided it is sealed to prevent evaporation.

- Touch 5-10 colonies of similar appearance with a loop and transfer to a tube of broth, or transfer a loopful of confluent growth of a pure culture to a tube of broth.

- After incubation at 35°C for 4-6 hours, compare the broth culture with the turbidity standard. This comparison can be made more easily if the tubes are viewed against a background of white paper with print of various sizes on it. Adjust by diluting the broth culture with sterile broth or saline.

- Inoculate the plates by dipping a sterile cotton swab into the inoculum. Remove excess inoculum by pressing and rotating the swab firmly against the side of the tube above the level of the liquid. Streak the swab all over the surface of the medium three times, rotating the plate through an angle of 60° after each application. Finally, apply the swab all round the edge of the agar surface. Leave the inoculum to dry for a few minutes at room temperature with the lid closed.

This method of inoculation should give nearly confluent growth.

Inoculum according to bacterial species:

- If broth is used, overnight cultures of enterobacteria should be diluted about 10^{-4} (1:10000).

- When plates are used, prepare the suspension as follows: dip a 0.01-ml loop into five colonies, transfer to 1 ml saline, dip another 0.01-ml loop in this suspension, transfer to 1 ml saline, and finally dip a third 0.01-ml loop in this suspension and transfer to 5 ml saline. Mix the suspension well. Flood the plates.

This method of inoculation should give semiconfluent growth.

Choice of antimicrobial

Only a limited and carefully selected number of antimicrobials should be included in the susceptibility test. The most appropriate ones are: ampicillin, TMP-SMX, nalidixic acid, pivmecillinam and ciprofloxacin (or another fluoroquinolone).

Procedure for use of antimicrobial discs

Any commercially available discs or tablets with the proper potency can be used. The disc potency recommended in WHO guidelines is shown in the table. Avoid using discs with different potencies, as these can give misleading results.

Discs prepared locally: If antimicrobial discs cannot be obtained commercially, they can be prepared locally. Buy or cut 6-mm filter paper discs pretested for absence of antibacterial property. Place them separately on the bottom of a sterile Petri dish. Pipet 20 µl of antimicrobial solution on each disc. Use an antimicrobial solution that will give the disc potency shown in the table. Dry the disc for about one hour at 35°C. The paper discs may also be placed on the inoculated agar surface prior to pipetting the appropriate antimicrobial solutions.

Storage: Stocks of antimicrobial discs should preferably be kept at -20°C; the freezer compartment of a home refrigerator is convenient. A small working supply of discs can be kept in the refrigerator for up to one month. On removal from the refrigerator, the containers should be left unopened at room temperature for about one hour. This procedure reduces the amount of condensation that occurs when warm air enters the cold container. If a disc-dispensing apparatus is used, it should have a tight-fitting cover and be stored in the refrigerator. It should also be allowed to warm to room temperature before opening. Antimicrobial tablets (except carbenicillin) are stable for at least four years at room temperature.

Application of antimicrobials: The antimicrobial discs or tablets may be placed on the inoculated plates in zones properly divided and marked on the back, using either a pair of sterile forceps, an antimicrobial disc dispenser, or a sterile needle tip.

Not more than seven discs (one in the centre, six 15 mm from the edge of the plate) can be placed on a plate.

Incubation: The plates should be placed in an incubator at 35°C within 30 minutes of their preparation. Do not incubate in an atmosphere of carbon dioxide. Do not stack more than two

plates high. Incubate overnight.

Measurement of inhibition zones

After overnight incubation the diameter of each inhibition zone (including the diameter of the disc) is measured and recorded in mm. The measurements can be made with a ruler on the under-surface of the plate without opening the lid. If the medium is opaque, the zone can be measured by means of a pair of callipers.

The end-point of inhibition is judged by the naked eye at the edge of the clear zone where growth starts. With sulfonamides and sulfamethoxazole-trimethoprim, however, slight growth occurs within the inhibition zone; such growth should be ignored.

Interpretation of zone sizes

The result of the susceptibility test, as reported to the clinician, classifies the microorganism in one of three categories:

- Susceptible (S): A microbe is called "susceptible" to a drug when the infection caused by it is likely to respond to treatment with this drug, at the usual dosage.

- Intermediate (I): The susceptibility of an organism is called "intermediate" when the infection is likely to respond to unusually high doses of the drug, or when the organism is located in a part of the body where the drug is concentrated (urine, bile, intestinal lumen, local application). The intermediate category also acts as a *buffer zone* and compensates for small technical errors in the performance of the procedure.

- Resistant (R): This term implies that the infection is not likely to respond to a given antimicrobial, irrespective of the dosage and of the location of the infection.

The translation of the zone of inhibition into a susceptibility category depends on whether an absolute or a comparative method has been used.

Absolute method: This is performed with as high a degree of standardization as possible (medium, inoculum, etc.). This implies that for each antimicrobial one inhibition zone always corresponds to one MIC-value. Moreover, by means of a regression line and fixed MIC-breakpoints between S, I, and R, it is possible to translate the inhibition zone of the organism tested into S, I, or R according to the recommendations of the manufacturer.

Comparative method: This is based on a comparison of the inhibition zone of an unknown strain with that of a control strain and is *less satisfactory* than the absolute method. The control strains should be included every day and treated exactly as the test strains using identical media and inoculum density (semiconfluent growth). The control strains and the test strains can even be inoculated on the same plate on each side of the same disc, thus providing a control for every disc. This method is especially recommended if the performance of the disc is very poor and variable. *E. coli* NCTC 10418 and *Staph. aureus* NCTC 6571 are recommended as control strains in the comparative method. If the inhibition zones are measured from the edge of the disc to the edge of the zone, the following interpretation is used:

- Susceptible: Zone size equal to, wider than, or not more than 3 mm smaller than the control.

- Intermediate: Zone size greater than 3 mm, but smaller than the control by more than 3 mm.

- Resistant: Zone size 3 mm or less.

Day-to-day controls

All susceptibility tests are very sensitive to small variations in media, inoculum, incubation, temperature, etc. In order to perform a reliable test it is of the utmost importance every day to include control strains in the test.

If absolute methods are used, the following control strains should be included daily: *Staph. aureus* (ATCC 25923) and *E. coli* (ATCC 25922).

In comparative methods, control strains are automatically included every day in order to interpret the inhibition zones of the unknown strains. Records should be kept of the inhibition zones of the control strains to detect large variations. A decrease in potency of the discs on storage may be revealed by a decrease in the size of the inhibition zone around a control strain.

The control strains for both the absolute and comparative tests can be obtained from the American Type Culture Collection[13] or other national culture collections. They are provided in the form of pellets of desiccated pure cultures. Cultures for day-to-day use should be grown on slants of nutrient agar (trypticase soy agar is convenient) and stored in the refrigerator. They should be subcultured onto fresh slants every two weeks. The control strains are treated just like the other pure cultures investigated by the susceptibility test, and the inhibition zones recorded.

When the procedure is correctly performed, the zone sizes shown by the control organisms should fall within the range of diameters given in the table. The limits that can be tolerated in the test have been determined in a collaborative study involving a large number of reputable laboratories, and reflect the degree of accuracy than can be routinely obtained by a good clinical laboratory. When results fall regularly outside this range, they should be regarded as evidence that one or more technical errors have been introduced into the test or that the reagents are at fault. Each reagent, and each step in the test, must be investigated until the cause of the error has been eliminated.

Grossly aberrant results that cannot be explained by technical errors in the procedure may indicate contamination or sudden changes in the susceptibility or growth characteristics of the control strain. If this occurs, a fresh control strain should be obtained from a reliable source.

[13] Major collections of type cultures:
1. American Type Culture Collection (ATCC), 12301 Parkland Drive, Rockville, MD 20852, USA
2. National Collection of Type Cultures (NCTC), Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, United Kingdom

ANNEX 9

Isolation and identification of *Escherichia coli* O157

Escherichia coli O157 is not detected by the usual methods used to isolate and identify traditional enteric bacterial pathogens. However, the isolation and identification of *E. coli* O157 can be performed by most laboratories, given the proper medium and antiserum.

Background

E. coli O157 ferment D-sorbitol slowly or not at all, unlike most other *E. coli* strains which ferment sorbitol rapidly. This finding led to the development of sorbitol-MacConkey agar, which is now commercially available. Sorbitol-negative colonies are colourless and are considered "suspicious for *E. coli* O157". Other *E. coli* colonies are pink to red.

Methods

Preparation of faecal suspension: See guidelines in Annex 7.

Plating on sorbitol-MacConkey agar: Each new batch of medium should be controlled for quality prior to routine use by inoculating it with known reference strains and testing for growth and colony characteristics. Streak one loopful of suspension onto the plate. Incubate 24 hours at 36°C. Sorbitol-negative colonies are colourless and are considered "suspicious for *E. coli* O157".

Agglutination: Strains of *E. coli* O157 rapidly agglutinate in O157 antiserum, which is commercially available. Select three isolated sorbitol-negative colonies for agglutination. Follow the manufacturer's instruction for the specific agglutination procedure. Once a positive colony is encountered, additional colonies need not be tested, and the specimen is considered "positive for *E. coli* O157".

If sorbitol-MacConkey agar is not used, 5-10 lactose-positive colonies from MacConkey agar or a similar plating medium can be screened in tubes of sorbitol fermentation medium. At least five colonies should be selected for screening because the organism is not always present in pure culture. Colonies that do not ferment sorbitol within 24 hours are screened for agglutination in *E. coli* O157 antiserum, as outlined above.

Isolated strains should be sent to a reference laboratory for confirmation and further typing.

ANNEX 10

Treatment supplies for 100 persons in dysentery epidemics

Sanitary/hygienic supplies

200 grams of hand soap per person per month
30 boxes soap for washing clothes
2 one-litre bottles of cleaning solution (2% chlorine or 1-2% phenol)

Rehydration supplies

100 packets ORS (for one litre each)
20 bags Ringer's lactate solution (one litre each)
5 scalp vein sets
10 adult IV giving sets

Antimicrobials

For adults: 400 one-gram tablets of nalidixic acid
For children: 400 one-gram tablets of nalidixic acid

Other antimicrobials may need to be substituted for nalidixic acid depending on local antimicrobial susceptibility patterns.

Other treatment supplies

One large water dispenser
5 one-litre bottles for ORS solution
5 half-litre bottles for ORS solution
10 tumblers
5 teaspoons

Assumptions:

- 20% of cases are children ≤5 years. All are treated with antimicrobials.
- 80% of cases are >5 years old. All are treated with antimicrobials.
- 20% of cases will have dehydration requiring ORS.
- 10% of cases will have dehydration requiring IV fluids.

- Each person will be given 200 grams of hand soap per month.
- Each family will be given soap for washing clothes and bed linen of ill person.

ANNEX 11

Feeding during and after diarrhoea

General guidelines

Encourage the child to eat during the entire illness

During and after illness, feed the child as follows:

Up to age 4-6 months

- Breastfeed as often as the child wants, day and night.
- For children taking other milk, give appropriate milk as often as the child wants by cup. Increase the breastfeed while gradually reducing the other milk over several days. Give the additional milk by cup, not by bottle
- If the child is fed on other milk alone, give this as often as the child wants by cup, not by bottle.

4-6 months up to 12 months

- Breastfeed as often as the child wants.
- Give foods three times a day if breastfed or five times a day if not breastfed

12 months up to 2 years

- Breastfeed as often as the child wants
- Give food five times a day

Above 2 years

- Give family foods: three meals + two additional feeds.

ANNEX 12

WHO collaborating centres for *Shigella*

The following two centres may be approached for technical assistance in bacteriological aspects of Shigella:

WHO Collaborating Centre for Shigella
Diarrheal Diseases Laboratory Section
Foodborne and Diarrheal Diseases Branch
National Center for Infectious Diseases
National Center for Disease Control
and Prevention (CDC)
Atlanta, GA 30333
USA

Tel. 1 404 639 3331
Fax. 1 404 639 3970

WHO Collaborating Centre for Phage-typing and Resistance of Enterobacteria
Division of Enteric Pathogens
Central Public Health Laboratory
Colindale Avenue
London NW9 5HT
United Kingdom

Tel. 44 181 2004400
Fax. 44 181 2007874

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