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Full Length Research Paper

Antibiotics susceptibility of a strain of *Salmonella* isolated from an infant presented with diarrhea in Ile-Ife, Nigeria

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Salmonella **was identified in the stool sample of a six months old baby presenting diarrhea and hospitalized at the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, Nigeria. The isolate was urease negative, citrate utilization positive, indole negative, methyl red positive but Voges-Proskaeur negative. It was motile, catalase negative and unable to produce hydrogen sulphide. It was able to ferment glucose with the production of acid and gas but unable to ferment lactose and sucrose. The organism was sensitive to amoxillin, cotramoxazole, nitrofurantoin, gentamicin, ofloxacin and tetracycline but resistant to nalidixic acid and augmentin.**

Key words: Antibiotics susceptibility, *Salmonella*, diarrhea.

INTRODUCTION

Diarrhea is a gastrointestinal infection defined as a change in the consistency of stool to being abnormally loose or fluid and an increase in the frequency of stool to more than its normal (Akinbami, 1999). It is an intestinal disorder characterized by abnormal fluidity and frequency of feacal evacuations. Generally, the result of increased motility in the colon may be an important symptom of such underlying disorders as dysenteric diseases, lactose intolerance, gastrointestinal tumors and inflammatory bowel diseases (Khan-Mohammed et al., 2005). World wide, diarrheal diseases are second only to respiratory diseases as a cause of adult mortality. They are the leading cause of childhood death in some parts of the world (Prescott et al., 2005). They contribute substantially to pediatric morbidity and mortality worldwide (Akinbami, 1999). According to the World Health Organization, estimates of 10 million children die from diarrheal diseases each year before their fifth birthday (Clarke et al., 2002). At birth, the intestine is normally sterile but organisms are later introduced with food. In breast-fed children, the intestine contains large numbers of lactic acid *Streptococci* and *Lactobaccilli.* In bottle-fed children, mixed flora exists in the bowel and *Lactobacillus* is less predominant (Brocks et al., 2003). The gastrointestinal tract of humans is the normal habitat for many different gram negative bacilli, most of which belong to the family *Enterobacteriaceae* (Graham et al., 2007). A number of genera within the family are human intestinal pathogens. These include *Shigella*, *Yersinia* and *Salmonella* (Brock and Madigan, 1999).

Salmonella are a group of motile rod-shaped bacteria living in the intestinal tracts of animals and birds. They cause intestinal illness (Salmonellosis) in humans and are usually acquired through ingestion of contaminated foods or drinks (Brock and Madigan, 1999). Both young and old with weakened immune systems from diseases such as AIDS or as a result of cancer treatments are most vulnerable (Pelczar et al., 1997). *Salmonella* possess a variety of antigens which form the basis for serological classification. The two major types of antigens associated with *Samonella* are the somatic antigens which are lipoprotein-polysaccharides associated with bacterial cell wall and the proteinous flagella antigens, associated with the flagella, used by the bacteria for movement (Pelczar and Reid, 1993). Non-motile mutants

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Table 1. Physical characteristics of samples.

Sample	Texture	Appearance
1	Semi solid	Non mucoid, non bloody
2	Semi solid	Non mucoid, non bloody
3	Semi solid	Non mucoid, non bloody
4	Semi solid	Non mucoid, non bloody
5	Liquid	Non mucoid, non bloody
6	Liquid	Mucoid
7	Semi solid	Non mucoid, non bloody
8	Liquid	Non muciod, non bloody
9	Liquid	Mucoid
10	Semi solid	Non mucoid, non bloody
11	Semi solid	Non mucoid, non bloody
12	Semi solid	Non mucoid, non bloody
13	Liquid	Bloody

Table 2. Biochemical reactions.

Test	Observation
Urease	Negative
Citrate	Positive
Indole	Negative
Methyl red	Positive
Voges-Proskaeur	Negative
H_2S production	Positive
Catalase	Negative
Motility	Positive
Gram stain	Gram negative bacilli

of *Salmonella* may also occur (Pelczar et al., 1997).

Prevalence of diarrheal infections in Ile-Ife, Nigeria has been particularly high in recent times. *Salmonella* has been detected in stool samples of a number of individuals in the community. The present study was designed to determine the antibiotics susceptibility pattern of a strain of this organism isolated from the stool sample of a six months old infant presenting diarrhea.

MATERIALS AND METHODS

Collection of samples

Stool samples were collected from patients, including a six month old infant having typhoid fever, presented with diarrhea and hospitalized at the Obafemi Awolowo University Teaching Hospital complex (OAUTHC), Ile-Ife Nigeria. The samples were collected, upon consent of patients, into universal bottles and labeled. Collection was under aseptic conditions to avoid contamination. They were brought to the laboratory for immediate analysis (Olutiola et al., 1991). The period of study was between June and December 2008.

Media

Media used were MacConkey agar, *Salmonella-Shigella* agar (SSA) and nutrient agar. They were of analytical grade and prepared according to manufacturer's specifications.

Biochemical tests and staining method

The biochemical tests carried out were motility, urease, Koser's citrate utilization, catalase, indole, hydrogen sulphide production, methyl red Voges-Proskaeur (MRVP) sugar fermentation tests and Gram staining.

Antibiotics susceptibility test

Antibiotics susceptibility was carried out on *Salmonella* positive isolate from the six months old infant using standard disc diffusion method. Diagnostic sensitivity agar (DST) (Mueller Hinton Agar) (Oxoid) was employed. Gram negative discs (Oxoid) were used. Zones of inhibition were measured and recorded.

Isolation of Salmonella

Samples were inoculated onto MacConkey agar plates and incubated at 37°C for 24 h. Non-lactose fermenters suspected to be *Salmonella* species were subcultured onto plates of Salmonella-Shigella agar (SSA). Incubation was at 37°C for 24 h. Black coloured colonies suspected to be *Salmonella species* were re-cultured onto SSA to obtain pure colonies. The pure colonies were further cultured onto nutrient agar slants for preservation and biochemical tests (Olutiola et al., 1991).

RESULTS

Thirteen stool samples were analysed in order to isolate and identify *Salmonella*. Five of the samples were liquid, eight were semi-solid and two mucoid in texture. One sample was bloody (Table 1). Five of the samples were positive for *Salmonella*.

The isolate from the six months old infant was found to be gram negative. Confirmation was pinkish red colour of the bacilli when viewed under oil immersion objective of a binocular microscope (Table 2).

The isolate was motile, confirmed by growth away from the line of stab of the motility test medium after incubation at 37°C for 7 days. Examination was on a daily basis (Table 2).

The isolate was able to utilize citrate as sole carbon source shown as the change in colour of the inoculated koser citrate medium from green to blue after incubation at 37°C for 5 days (Table 2).

The isolate was catalase negative. No bubbles were evolved when hydrogen peroxide was added to the growth (Table 2).

The isolate was indole negative indicated by the absence of colour change to red when Kovac's reagent was added to the inoculated test medium after 7 days incubation at 37°C (Table 2).

After 5 days incubation at 37°C, the isolate was observed to be methyl red positive but Voges-Proskaur negative (Table 2).

Table 3. Sugar fermentation test.

Test	Observation
Lactose	Nil
Sucrose	Nil
Glucose	AG

A, Acid production; G, Gas production; AG, Acid and gas production; Nil, No acid, no gas production.

Table 4. Antibiotics susceptibility of Salmonella isolated from stool sample of a six month old infant.

Antibiotics	Concentration (µg)	Reaction
Amoxillin	25	S
Cotramoxazole	25	S
Nitrofurantoin	300	S
Gentamicin	10	S
Nalidixic acid	30	R
Ofloxacin	30	S
Augmentin	30	R
Tetracycline	30	S

R, Resistant; S, Sensitive.

The isolate was able to ferment glucose indicated by the production of acid and gas (colour changes from red to yellow and the presence of bubbles in inverted Durham tubes). It was unable to ferment lactose and fructose (Table 3).

The isolate was resistant to augmentin and nalidixic acid but susceptible to amoxillin, cotrimoxazole, nitrofurantoin, gentamicin, ofloxacin and tetracycline (Table 4).

DISCUSSION

Salmonella are ubiquitous pathogens of humans and animals (Brock and Madigan, 1999). *Salmonella* was isolated from some of the feacal samples of individuals having diarrhea from the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, Nigeria.

Several factors may be responsible for the presence of *Salmonella* in feacal samples. The consumption of contaminated food or drinks is one of such factors. Contamination could also be through improper handling of food or drinks, consumption of untreated water from wells, streams, rivers and boreholes.

Most individuals infected with *Salmonella* develop diarrhea, fever and abdominal cramp12-72 h after ingestion (Brooks et al., 2003). Salmonellosis ranges, clinically, from the common Salmonella gastroenteritis (diarrhea, fever and abdominal cramp) to enteric fever (including typhoid fever) which are life- threatening febrile systemic illnesses requiring prompt attention and antibiotic therapy (Centre for Disease Control and Prevention, 2003).

Five out of the thirteen samples were positive for *Salmonella* on the SSA media. The diagnosis of salmonellosis requires bacteriological isolation of the organism from appropriate clinical specimen and confirmation through biochemical procedures. Serological tests which include the identification of both the somatic and flagella antigens of Salmonella can be further carried out (Gruenewald et al., 1999). Two main species of Salmonella: *Salmonella typhii and Salmonella paratyphii* are frequently encountered in these regions.

The species isolated from the six months old infant was susceptible to amoxillin, cotramoxazole, nitrofurantoin, gentamicin, ofloxacin and tetracycline but resistant to nalidixic acid and augmentin.

Antibiotics resistance could result from indiscriminate use of antibiotics. The organism develops resistance plasmids which can be transferred, by mutation, to subsequent generations of the organism (Bastarrachea, 1998; Cochella and Green, 2004).

Awareness should be an important component of health education on food and water borne infections in these regions. People should also be educated on personal and environmental hygiene. Proper treatment of consumed water should be imparted, most especially, to rural dwellers who have limited access to chlorine treated, pipe-borne water. Education and awareness at the primary, secondary and tertiary institutions of learning should be paramount.

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