

Full Length Research Paper

Characterization and recovery rates of food-indicator microorganisms from home-made oral rehydration solutions in Nigeria

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From home-made oral rehydration solutions (ORS), the identified bacterial strains from a total of 1880 bacterial isolates (1010 from granulated sugar and 870 from table salt) using the conventional taxonomic tools were *Bacillus cereus* var. *mycoides* (0.57%), *Bacillus subtilis* (2.28%), *Citrobacter* sp. (1.07%), *Clostridium perfringens* (14.75%), *Enterobacter aerogenes* (6.13%), *Escherichia coli* (7.44%), *Klebsiella pneumoniae* (10.0%), *Morganella morganii* (0.78%), *Proteus mirabilis* (6.74%), *P. vulgaris* (1.68%), *Pseudomonas aeruginosa* (4.67%), *Salmonella enterica* serovar Typhi (3.89%), *Salmonella enterica* serovar Typhimurium (0.99%), *Shigella dysenteriae* (11.0%), *Staphylococcus aureus* (11.98%) and *Vibrio cholerae* (2.57%). The isolated fungal species from the table salt and granulated sugar samples were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Botryodiplodia* sp., *Candida* sp. and *Scopulariopsis* sp. Home-made ORS may serve as a means of transmitting gastroenteritis/diarrhoea and other infectious microbial agents in developing countries like Nigeria

Key words: Characterization, food indicator organisms, home-made, oral dehydration solutions, recovery rates.

INTRODUCTION

Diarrhoeal disease is one of the most important causes of morbidity and mortality in infants and young children in the less developed world. In an extremely conservative estimate, investigators calculated that there are at least 750 million to 1 billion episodes of diarrhoea and 4.6 million deaths each year due to diarrhoea in children less than 5 years of age in Africa, Asia (excluding the People's Republic of China), and Latin America. They also estimated that approximately 1 in every 167 episodes of diarrhoea in young children results in death (WHO, 1990, 1995; UNICEF, 1995).

Measures of child health are useful indicators of the health of a nation, particularly in Nigeria where children constitute about 45% of the total population, and the country's infant mortality rate of 114 per 1000 live births

is among the highest in sub-Saharan Africa and mortality among children under five years of age is as high as 300 per 1000 live births in some parts of the country, while epidemiological evidence also shows that diarrhoea is a major problem, with an estimated one-in-six children under the age of five years experiencing at least one episode every fortnight (FOS, 1997).

Diarrhoea causes a loss of body water and salts (electrolytes), which, if sufficiently copious and not adequately replaced, may result in dehydration. Approximately 1 of every 150 to 200 episodes of infant diarrhoea results in severe, life threatening dehydration. Irrespective of the specific infectious agent causing diarrhoea, the treatment of diarrhoeal dehydration is the same and involves the replacement of body water and electrolytes (salts in solution). This observation has allowed the development of oral rehydration therapy (ORT) – a highly efficacious form of therapy for diarrhoeal dehydration that is technologically appropriate for use in less developed countries. ORT, which involves oral

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rehydration with solutions containing glucose or sucrose and electrolytes, now is in use worldwide, in programs sponsored by international agencies and national governments.

It is not, however, possible economically or logistically to provide a packet of balanced sugar/electrolyte powder to treat every episode of diarrhoea in all young children in developing countries. For that reason, some observers have advocated the use of simple dehydration solutions of table salt and sugar, prepared and administered in the home (Anonymous Development, 1981; Kilemann and McCord, 1997; Levine et al., 1981; PIP, 1980). Several methods have been devised for the preparation of simple sugar/salt solutions that are safe and can be prepared in the home. These simple solutions are just as effective as more complex balanced glucose/electrolyte solutions in stimulating sodium and water absorption by the intestine (Levine et al., 1981).

The primary focus of this study is therefore on home-prepared oral rehydration solutions (ORS). The discussion that follows review the basic scientific contributions that provide a microbiological basis for this therapy, obstacles that had to be overcome to give it widespread acceptance, and hurdles that are now having to be passed in order to realize the implementation of home-prepared ORT on a global scale especially in a developing country like Nigeria.

MATERIALS AND METHODS

Sampling

Five hundred packaged granulated sugar and three hundred and seventy packaged table salt samples obtained from the Federal capital territory, Abuja; five southwestern states- Lagos, Ogun, Oyo, Osun, Ekiti and Kogi state (a middle belt state) of Nigeria between February, 2001 and July, 2004 were microbially analyzed in the laboratory to determine their microbial content.

Isolation of the microbial flora of the samples

The overnight broth culture (1 ml) of each table salt and granulated sugar samples in alkaline peptone water (pH 8.6) was transferred into sterile plates by plating decimal dilutions of each sample in triplicates with different molten agar at 45°C. These include nutrient agar (NA; LAB M), *Salmonella-Shigella* agar (SS; LAB M), thiosulphate citrate bile sucrose (TCBS; Oxoid) agar, pH 8.2; mannitol salt agar (MSA; LAB M), MacConkey agar (Oxoid), (LAB M) at pH 7.4, cysteine lactose electrolyte deficient (CLED; LAB M) and Sabouraud dextrose agar (SDA; LAB M). The culture dilutions were aseptically added to the plates and incubated between 24-48 h at 35°C for bacterial isolation and at 25°C for fungal isolation according to the methods of Cruickshank et al. (1975). The population, in colony-forming units (CFU), and the characteristics of the colonies were recorded for each medium.

Purification and preservations of the isolates

Representatives of each different bacterial colony types were randomly picked from the primary plates of each sample and sub-

cultured onto sterile plates by the streaking method. The isolates were sub-cultured by repeated streaking to obtain pure cultures. All the bacterial isolates were kept at 4°C in triplicates, on Brain Heart Infusion (BHI) agar slants as working and stock cultures.

Characterization and identification of the isolates

Taxonomic studies were carried out on the purified isolates from the differently analyzed samples on the basis of their cultural, morphological, biochemical and physiological characteristics. Tentative identification of the bacterial species was based on the conventional identification characteristics of the strains while the general key used for the identification was by reference to Kloos and Schleifer (1975), Harrigan and McCance (1976) and Bergey's Manual of Systematic Bacteriology (1974).

RESULTS

The bacterial counts of the granulated sugar samples were between 1.0×10^3 cfu ml⁻¹ (log 3.0 cfu ml⁻¹) and 4.6×10^3 cfu ml⁻¹ (log 3.66 cfu ml⁻¹) on MacConkey agar plates. About 18% of the granulated sugar samples had bacteria too numerous to count (TNTC) colonies while 5% had swarming colonies on MacConkey agar plates. On mannitol salt agar plates, the bacterial loads of the granulated sugar samples were between 1.0×10^3 cfu ml⁻¹ (log 3.0 cfu ml⁻¹) and 3.0×10^3 cfu ml⁻¹ (log 3.48 cfu ml⁻¹). On plate count agar, the colonial counts were between 3.6×10^4 cfu ml⁻¹ (log 4.56 cfu ml⁻¹) and 5.2×10^4 cfu ml⁻¹ (log 4.72 cfu ml⁻¹) (Table.1) while on thiosulphate citrate bile sucrose agar (TCBS) plates the bacterial counts were between 1.0×10^3 cfu ml⁻¹ (log 3.0 cfu ml⁻¹) and 2.1×10^3 cfu ml⁻¹ (log 3.32 cfu ml⁻¹).

The bacterial counts of the table salts on MacConkey agar plates were between 1.0×10^3 cfu ml⁻¹ (log 3.0 cfu ml⁻¹) and 5.3×10^3 cfu ml⁻¹ (log 4.72 cfu ml⁻¹). Twenty percent of the samples had TNTC colonies while 5.0% of had swarming colonies on MacConkey agar plates. On mannitol salt agar plates, the bacterial loads of the table salt samples were between 1.0×10^3 cfu ml⁻¹ (log 3.0 cfu ml⁻¹) and 2.5×10^3 cfu ml⁻¹ (log 3.40 cfu ml⁻¹). On plate count agar, the colonial counts were between 5.8×10^4 cfu ml⁻¹ (log 4.76 cfu ml⁻¹) and 8.1×10^4 cfu ml⁻¹ (log 4.91 cfu ml⁻¹) while on thiosulphate citrate bile sucrose agar (TCBS) plates, the bacterial counts were between 4.6×10^4 cfu ml⁻¹ (log 4.66 cfu ml⁻¹) (Table.2) and 6.1×10^4 cfu ml⁻¹ (log 4.79 cfu ml⁻¹).

The results of the total coliform test (37°C) gave 37.3% of the granulated sugar samples to be total coliform positive at 24 h and 49.0% to be total coliform positive at 48 h while 3.7% of the table salt samples were total coliform positive at 24 h and 8.11% were total coliform positive at 48 h of incubation.

A total of 1880 bacterial isolates were obtained from the samples analyzed in this study. 1010 bacterial isolates from granulated sugar samples and 870 bacterial isolates from table salt samples were recorded in this study.

Table 1. Distribution and frequency of occurrence of the bacterial species isolated from table salt and granulated sugar samples.

Bacterial species	Sources and frequency of occurrence	
	Table salt	Granulated sugar
<i>Bacillus cereus</i> var. <i>mycoides</i>	5 (0.57)	nd
<i>B. subtilis</i>	20 (2.28)	nd
<i>Citrobacter</i> sp.	10 (1.14)	10 (0.99)
<i>Clostridium perfringens</i>	162 (18.6)	110 (10.9)
<i>Enterobacter aerogenes</i>	81 (9.3)	30 (2.97)
<i>Escherichia coli</i>	52 (5.97)	90 (8.91)
<i>Klebsiella pneumoniae</i>	81 (9.3)	290 (28.7)
<i>Morganella morganii</i>	5 (0.57)	10 (0.99)
<i>Prot. mirabilis</i>	57 (6.55)	70 (6.93)
<i>Prot. vulgaris</i>	12 (1.38)	20 (1.98)
<i>Pseudomonas aeruginosa</i>	123 (14.1)	80 (7.92)
<i>Salm. enterica</i> var. Typhi	7 (0.85)	70 (6.93)
<i>Salm. enterica</i> var.	nd	10 (0.99)
Typhimurium		
<i>Staphylococcus aureus</i>	148 (17.0)	70 (6.93)
<i>Shigella dysenteriae</i>	71 (8.16)	140 (13.9)
<i>Vibrio cholerae</i>	36 (4.14)	10 (0.99)
Total no of isolates	870	1010

nd = not detectable limit on culture media. Values in parenthesis represent the percentage occurrence of the isolated bacterial species.

The identified bacterial species from the granulated sugar samples were (Table 1) *Citrobacter* sp. (0.99%), *Clostridium perfringens* (10.9%), *Enterobacter aerogenes* (2.97%), *Escherichia coli* (8.91%), *Klebsiella pneumoniae* (28.7%), *Morganella morganii* (0.99%), *Proteus mirabilis* (6.93%), *P. vulgaris* (1.98%), *Pseudomonas aeruginosa* (7.92%), *Salmonella enterica* serovar Typhi (6.93%), *Salmonella enterica* serovar Typhimurium (0.99%), *Shigella dysenteriae* (13.9%), *Staphylococcus aureus* (6.93%) and *Vibrio cholera* (0.99%) (Table 1).

The identified bacterial species from table salt samples however were *Bacillus cereus* var. *mycoides* (0.57%), *Bacillus subtilis* (2.28%), *Citrobacter* sp. (1.14%), *Clostridium perfringens* (18.6%), *Enterobacter aerogenes* (9.3%), *Escherichia coli* (5.97%), *Klebsiella pneumoniae* (9.31%), *Morganella morganii* (0.57%), *Proteus mirabilis* (6.55%), *P. vulgaris* (1.38%), *Pseudomonas aeruginosa* (14.1%), *Salmonella enterica* serovar Typhi (0.85%), *Shigella dysenteriae* (8.16%), *Staphylococcus aureus* (17.0%) and *Vibrio cholera* (4.14%) (Table 1).

The recovery rates of the isolated bacterial species indicated that *Staphylococcus aureus*, *Clostridium perfringens*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *E. coli*, *Klebsiella pneumoniae* and *Proteus* species from table salt samples while *Klebsiella pneumoniae*, *Clostridium perfringens*, *E. coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* and

Staphylococcus aureus were the most commonly isolated bacterial species from granulated sugar samples.

Table 2. Distribution and frequency of occurrence of the bacterial species isolated from table salt and granulated sugar samples.

Bacterial species	Sources and frequency of occurrence	
	Table salt	Granulated sugar
<i>Aspergillus flavus</i>	181 (20.8)	124 (12.3)
<i>Aspergillus fumigatus</i>	121 (13.9)	90 (8.91)
<i>Aspergillus niger</i>	93 (10.7)	140 (13.9)
<i>Botryodiplodia</i> sp	32 (3.68)	41 (4.06)
<i>Scopulariopsis</i> sp	12 (1.38)	20 (1.98)
<i>Candida</i> sp.	263 (30.2)	148 (14.6)
Total no of isolates	702	563

Values in parenthesis represent the percentage occurrence of the isolated bacterial species.

Clostridium perfringens was recovered from 80 (21.6%) of the table salt and 83 (16.6 %) of the granulated sugar samples; (Table 2) *Pseudomonas aeruginosa* was recovered from 55 (14.9%) of the table salt and 68 (13.6%) of the granulated sugar samples; *Staphylococcus aureus* was recovered from 84 (22.7%) of the table salt and 51 (10.2%) of the granulated sugar samples; *Shigella dysenteriae* was recovered from 53 (14.3%) of the table salt and 68 (13.6%) of the granulated sugar samples. *Escherichia coli* was recovered from 48 (13.0%) of the table salt and 71 (14.2%) of the granulated sugar samples; *K. pneumoniae* was recovered from 39 (10.5%) of the table salt and 86 (17.2%) of the granulated sugar samples while *Proteus mirabilis* was recovered from 34 (9.19%) and 25 (5.0%) of the granulated sugar samples.

The isolated fungal species from the table salt and granulated sugar samples were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Botryodiplodia* sp., *Candida* sp. and *Scopulariopsis* sp. (Table 2).

DISCUSSION

Up to the last decade, there was no simple and effective intervention to attack the vicious cycle involving infant diarrhoea. However, such an intervention now exists: ORT. This is an inexpensive, highly effective means of replacing the deficits of body water and electrolytes in dehydrated infants that is technologically appropriate for use in less developed countries. ORT constitutes a revolutionary innovation in the treatment of diarrhoeal disease that has the potential to greatly diminish infant mortality throughout the world, but because of the lesser expense and greater availability of sucrose (table sugar), trials have been carried out to compare the efficacy of sucrose/electrolyte versus glucose/electrolyte solutions (Black et al., 1981; Nalin et al., 1978; Palmer et al., 1977;



Figure 1. Map of Nigeria showing the samples' collection points within the country. 1 = Abuja; 2 = Lagos, 3 = Ogun, 4 = Oyo, 5 = Osun, 6 = Ekiti, 7 = Kogi, 8 = Benue 9 = Edo, 10 = Ondo, 11 = Kwara.

Sack, et al., 1978, 1980) so that if economic or logistic considerations are paramount, sucrose-based solutions can be routinely used with expectation of excellent clinical results.

Based on the conventional phenotypic taxonomic tools, the *Salmonella enterica* strains isolated in this study were characterized as *S. enterica* serovar Typhi, and *S. enterica* serovar Typhimurium, in accordance with the proposition of LeMinor and Popoff (1987) that *S. enterica* be used as the species name. Varnam and Evans (1991) stated that the use of species-like epithets for serovars is a practice unique to *Salmonella*, and stems from the early recognition of the importance of the organism in pathology.

The distinguishing cultural and Biochemical characteristics of the isolated *Shigella* species on culture media were in conformity with those reported by Cowan and Steel (1974), Jones (1988) and Smith et al. (1990). *Citrobacter* sp., *Enterobater aerogenes*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* are type genera of the family Enterobacteriaceae, and share common characteristics of that family. They are the other species of Gram-negative bacteria isolated from this study Their cultural morphologies, Gram's identities and biochemical characteristics were also in agreement with

those of Buchanan and Gibbons (1974), Varnam and Evans (1991), Brooks et al. (1998) and Prescott et al. (2005). *Bacillus* and *Staphylococcus* were the only genera of Gram-positive bacteria isolated in this study.

The characteristics determined in identifying the species share similar profiles with previous works of Kloos andSchleifer (1975), Prescott et al. (2005) and Ogunshe Schleifer (1975), Prescott et al. (2005) and Ogunshe (2004).

In Nigeria, the most common and nationally advocated method of preparing simple sugar/salt solutions (ORS) is dissolving measured quantities of table salt and sugar (sucrose) in cool boiled water. (Figure 1) However, the results obtained in this study indicating that all the pathogens recovered in high microbial quantities from the constituents (salt/sugar) of ORS may be hazardous, especially in infants and children, since the pathogens have been implicated in infection such as gastroenteritis by previous studies of Simon et al. (1990), Lovet (1998), McKillip (2000), CDC (2002), Liu et al. (2003), Chern et al. (2004), Ogunshe (2004).

According to Anon (1986) diarrhoeal episodes of infective aetiology represent around 27% of those reported and *Shigella* species are among the five most frequently identified pathogens in children with acute

diarrhoea or dysentery, leading to a number of serious complications and high mortality rates. Thomas and Tillet (1973) had earlier also stated that shigellosis gets little press coverage but has a high mortality rate in developing countries, especially *Shigella flexneri*, even when treatment is available.

The genus *Salmonella* has been reported to be mostly associated with juvenile gastroenteritis in many countries (Tirado and Schmdit, 2001), while it was reported by Prescott et al. (2005) that *Salmonella* gastroenteritis (salmonellosis) is caused by over 2,000 *Salmonella* serovars. Chen et al. (2004) also reported that *E. coli* plays a role as diarrhogenic pathogens in infants. According to Lovet (1998) and Liu et al. (2003), the frequency of *E. coli* infection has led demand for therapeutics to treat acute *E. coli* infections. In 1996, a large outbreak involving more than 6,000 primary school children was reported by Liu et al. (2003) to have occurred in Sakai, Osaka, Japan.

Other enterotoxigenic gastroenteritis- causing genera such as *Pseudomonas*, *Enterobacter*, *Klebsiella* and *Proteus* isolated from table salt and granulated sugar samples in this study have been previously reported by Back et al. (1980) and Jiva et al. (1988) in infantile gastroenteritis. *Pseudomonas aeruginosa* was implicated in infantile gastroenteritis transmitted through water and foods by Rokoszewska et al. (1980) and Klipstein and Engert (1976) also implicated *Enterobacter* sp. as an opportunistic pathogen in extra-intestinal infections associated with diarrhoea in children.

Klebsiella spp. are also recognized as being opportunistic pathogens and have become of increasing importance. One species, *Klebsiella pneumoniae*, has been implicated as well by Klipstein et al. (1977) as a cause of diarrhoea while *Citrobacter* sp. was also established by Sakazaki (1984) as an opportunistic pathogen, and its role in diarrhoeal disease has been suggested by Guerrant et al. (1976) and Guarino et al. (1980). *Proteus mirabilis* and *Morganella morganii* (*Pr. Morganii*) have both been reported as well to be associated with diarrhoeal disease by Back et al. (1981). *Bacillus cereus* is a bacterium known to cause gastrointestinal infection, which is characterized by diarrhoea (McKillip, 2000; Phelps and Mckillip, 2000) while *Staph. aureus* has also been implicated in gastrointestinal illness by earlier findings of workers such as Sears and Kaper (1996) and Brooks et al. (1998).

As found out in this study the isolated fungal species from the packaged table salt and granulated sugar samples were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Botryodiplodia* sp., *Candida* sp. and *Scopulariopsis* sp., and this indicates that molds are common contaminants of the packaged table salt and granulated sugar samples. The presence of mycotoxins in food samples is of great concern for human health, more especially since many molds produce mycotoxins. *Aspergillus* species for example are known to be widely

distributed in nature and mostly produce aflatoxins, which are highly toxic metabolites produced by *Aspergillus* species. *Candida* species have also been known to cause thrush in children (Prescott et al., 2005). Since the mode of preparation of ORS in Nigeria does not involve any pre-treatment of the constituents (granulated sugar and table salt) by cooking, the toxic potential of home-made ORS is thus high.

Diarrhoeal disease is one of the most important causes of morbidity and mortality in infants and young children in the less developed world while dehydration is the most common precipitations cause of death in such infant diarrhea. The significance of ORS is to alleviate morbidity and mortality through fluid loss during gastroenteritis / diarrhoea (Hirschhorn, 1980; Hirschhorn et al., 1972; Hirschhorn and Greenough, 1991) However, the high recovery rates of the genera of bacteria isolated from table salt and granulated sugar samples, more especially from laboratory-prepared homemade ORS solutions as obtained in this study, should therefore of great concern. It is very obvious that the home-made ORS may also serve as a means of transmitting gastroenteritis/diarrhoea and other infectious microbial agents in developing countries like Nigeria if adequate care is not taken.

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