Bacterial profiles and consumer preference of some indigenous orally consumed herbal medications in Nigeria

Adenike A. O. Ogunshe *PhD*[1], Taiye R. Fasola *PhD*[2], and A. Egunyomi *PhD*[2]

[1]Applied Microbiology and Infectious Diseases Unit[2] Department of Botany & Microbiology, University of Ibadan, Ibadan, Nigeria
[2]Department of Botany & Microbiology, University of Ibadan, Ibadan, Nigeria

Abstract

Although consumer preference for indigenous herbal medications in Nigeria is on the increase, associated microbial hazards have not been documented. This study determined bacterial contents and consumer preferences of such medications. The isolated bacterial species from the indigenous herbal medications assayed from 1998 to 2004 were significant with 24 species identified. Bacteria survival rates in herbal concoctions ranged from 6 weeks to 12 months. Consumer preference for herbal medications in this study showed that 81.2 % of respondents had consumed herbal medicines and 77.5 % affirmed the potency of herbal medications. This study is clinically significant because most indigenous Nigerians are consuming herbal medications that are highly contaminated with potentially pathogenic bacterial flora due to poor quality control and preparation standards.

Keywords: bacterial profile, consumer preference, herbal medication indigenous, oral

Introduction

Africa is reputed for the extraordinary richness of its flora, which totals tens of thousands of species. Alternative medicines, such as herbal medicines, are gaining in popularity because of typically low side-effect profiles (Wilt et al. 2000), low cost (Vanderhoof 2001), and a high level of acceptance by patients and the majority of the population. Some managed care organizations now offer these therapies as an expanded benefit (Langyan and Ahuja 2005). In Africa, traditional medicine has always been a part of the culture even though this form of medicine is not as well organized as, for example, in India and China.

Herbal remedies are perhaps the most common form of alternative medicine. In fact, almost every nation or people have at one time used herbs and preparations of various sorts to treat illnesses and diseases. However, despite the use of herbs in medicine throughout the centuries, only a relatively small number of plant species and their extracts have been carefully studied (Tyler 1996; Awake 2000) and there is a minimal amount of information available on their safety and efficacy. The majority of information about herbs is usually based on anecdotal evidence and historical use.

On September 30, 1992, the government of Nigeria promulgated the Medical, and Dental Practitioners (Amendment) Decree No. 78, which placed natural medicine (traditional and alternative medicine) side by side with the orthodox system. Since then, there has been a high level of rivalry and advocacy against orthodox medicine by the traditional/herbal medical practitioners. Advertising in various forms by the herbal practitioners is unparalleled in Nigeria. People now attend hospitals as often as they go to herbalists (Okunade 2001), unfortunately, a large number of the herbal practitioners are illiterate, thus the publication of sensational and often misleading news on health related issues in media is alarming.

The main objectives of this research study are to determine the microbial quality of the indigenous herbal preparations packaged for human consumption for adults, infants and children in Nigeria and other parts of the world and to also investigate the consumer preference of herbal medications by Nigerians.

Materials and methods

An extensive research study was carried out between August 1998 and January 2005 to determine the microbial flora of about 250 locally prepared traditional medicines/concoctions. The herbal preparations were purchased from the herbal manufacturers at various herbal trade fairs and from certain local herbal manufacturers in Delta, Lagos, Ogun, Osun, Ondo and Oyo states of Nigeria between 1998- 2001 and 2002-2004.

Bacterial isolates

Overnight broth culture (1 ml) of each herbal samples in alkaline peptone water (pH 8.6) was transferred into sterile plates by plating decimal dilutions of each sample in triplicates, and molten

(45°C) nutrient agar (NA; 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, cystein lactose electrolyte deficient (CLED; LAB M) and violet red bile glucose agar (VRBG; LAB M) were aseptically added to the plates and incubated between 24-48 h at 35° C for the bacterial isolation (Cruickshank et al. 1975).

Representatives of each different bacterial colony type 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) as working and stock cultures.

The cultural characteristics of the purified isolates were described as observed on the different culture media used. Colonies developing on the plates were thus tentatively grouped on the basis of their colonial morphologies (Bailey and Scott 1974; Prescott et al. 2005).

Taxonomic studies were carried out on the purified isolates from the different herbal samples and tentative identification of the bacterial species was based on the microscopic, biochemical and physiological characteristics of the strains while the general key used for the identification was by reference to Kloos and Schleifer (1975) and Bergey's Manual of Systematic Bacteriology (Buchanan and Gibbons 1974).

Study on consumer preference of herbal medications

Questionnaire administration was employed for the determination of consumer preference of herbal medications. The questionnaires were designed solely for the purposes of this study and the questions were clear, relevant and unambiguous for easier understanding by the respondents. Respondents that were willing to partake in the study were administered with structured questionnaires designed to note the socio-cultural status and how familiar the respondents were with ethno-therapy; however, the questionnaires were only administered when the respondents were ready to fill in the questionnaires. The questionnaires were administered within four western states of Nigeria- Lagos, Ogun, Oyo and Osun but the respondents were selected to cut across the three major tribes of the country. Each respondent was asked same questions in interview format 3-5 days after collection of the questionnaire. Questionnaires that were not properly filled or having conflicting responses to same questions in both questionnaires and interview were discarded. A total of 1105 questionnaires were administered, however, 63 of the questionnaires were not returned/filled while 31 of the respondents were not available or gave a different response in the interview. Eight of the remaining 11 questionnaires were discarded because responses were not recorded for important questions. For purpose of approximation, the remaining 3 questionnaires were also not analysed.

Results

The total microbial loads of the indigenous orally consumed herbal medicines are indicated in Table 1. The bacterial colonies on plate count agar (PCA) were too numerous to count at 10^3 ml^{-1} while countable colonies were recorded on other culture media, with the lowest colony forming units recorded in eosin methylene blue (EMB) agar. Isolated microorganisms from the herbal samples are shown in Table 2. *Staphylococcus aureus* (29.80%), *Citrobacter aerogenes* (8.70%), *Pseudomonas aeruginosa* (7.83%), *Escherichia coli* (7.70%), and *Morganella morganii* (7.60%), were the most isolated bacterial species between 1998 and 2001 while *Klebsiella pneumoniae* (16.00%), *Morganella morganii* (15.00%), *Enterobacter aerogenes* (12.00%), *Pseudomonas aeruginosa* (11.20%), and *Escherichia coli* (9.00%) were the most isolated between 2002 and 2004. Bacterial isolates were recovered from all the herbal medications but with varying recovery rates. Out of the 133 herbal samples microbially analysed between 1998 and 2001, the recovery rates of the isolated bacterial species were *E. coli* 112 (84.2%); *Staph. aureus* 53 (39.8%); *Citrobacter aerogenes* 41 (30.8%); *Pseudomonas aeruginosa* 28 (21.0%) and *Morganella morganii* 21 (15.8%), while the recovery rates from 117 herbal remedies analysed between 2002 and 2004 were *E. coli* 82 (70.0%); *Enterobacter aerogenes* 49 (41.9%); *Pseudomonas aeruginosa* 56 (47.9%); *Klebsiella pneumoniae* 97 (82.9%) and *Morganella morganii* 21 (17.9%). The survival rates of the bacterial isolates in the herbal concoctions as determined in this study were between 6 weeks and 12 months.

Table 1: Colony counts of the bacterial isolates from herbal samples on different culture media.

Culture media (agar)	Colony counts cfu ml-1
Plate count (PCA)	too numerous to count at 10^3
MacConkey (MCC)	1.5×10^3 - 2.8×10^3
Cysteine lactose electrolyte deficient (CLED)	3.7×10^2 - 4.1×10^3
Eosin- methylene blue (EMB)	1.4×10^2 - 2.5×10^2
Thiosulphate citrate bile sucrose agar (TCBS)	7.2×10^2 - 9.6×10^2
Blood agar (BA)	5.6×10^2 - 8.3×10^2
Mannitol salt agar (MSA)	1.03×10^2 - 2.1×10^3

Table 2: Distribution of identities and frequency of occurrence of the bacterial species isolated from herbal samples.

Bacterial species	% Recovery of isolates	
	1998-2000	2001-2004
B. cereus	2.56	1.60
B. licheniformis	-	5.80
B. subtilis	5.13	-
Citrobacter aerogenes	8.70	6.60
C. freundii	2.56	-
Clostridium perfringes	1.30	-
Enterobacter aerogenes	6.41	12.00
Enterobacter cloacae	-	1.60
Escherichia coli	7.70	9.00
Klebsiella aerogenes	-	0.80
Klebsiella pneumoniae	2.56	16.00
Morganella morganii	7.60	15.00
Proteus mirabilis	1.30	0.80
Proteus retggeri	1.30	-
Proteus vulgaris	2.56	0.80
Pseudomonas aeruginosa	7.83	11.20
Salmonella var. typhi	1.30	-
Salmonella var. typhimurium	-	4.10
Serratia liquefaciens	2.56	-
Shigella dysenteriae	1.30	4.90
Shigella flexneri	-	5.79
Staphylococcus aureus	29.80	0.80
Streptococcus sp	-	0.80
Yersinia enterocolitica	2.40	2.40
Vibrio cholerae	5.13	-
% of isolates	100.00	99.99

Table 3: Percentage age distribution status of respondents

Age	<16	16-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	>64
%	2.9	0.0	30.4	16.7	12.6	18.7	6.3	6.3	0.0	3.7	1.3	1.1

The consumer preference study of 230 (23.0%) males and 770 (77.0%) females on herbal remedies indicated various opinions, observations and suggestions (Table 3). The ages of the respondents used in this study through questionnaire administration and verifying oral interview are as presented in Table 3. As a form of primary treatment for diseased condition, 79.9% of the respondents claimed to indulge in self-medication; 19.4% depended only on prescribed medication while 0.7% of the respondents did not indicate the nature of their primary treatment in cases of illness. Between 1998-2001, 10.0% of the respondents took herbal remedies; 50.0% took orthodox medications; 2.0% combined orthodox and herbal remedies while 38.0% did not indicate their medical status. However, between 2002-2004 29.0% of the respondents took herbal remedies; 21.0% took orthodox medications; 43.0% combined

orthodox and herbal remedies while 7.0% did not indicate their medical status during illnesses. Preference of respondents for herbal medications were given by 76.0% of the respondents, while a total of 77.5% of the respondents affirmed the potency of herbal medications. A least 81.0% of the respondents had taken herbal medications at one time or the other while 43.0% had taken herbal medications at least 5 or more times. 52.1% claimed to have had side effects from consumption of herbal medications, 21.3% stated that they never had any side effect from taking herbal medications, however, 26.6% did not respond. About half of the respondents (47.9%) placed the reason for their non-preference of herbal medications on the lack of prescriptions and the unhygienic nature of the herbal medications. Among the total respondents, only 35.5% suggested that all herbal medications should go through scientific research while just 25.0% wanted the herbal medications to pass through government regulations and registration. Among the total respondents, 43.7% claimed to have obtained information about herbal medicines from parents and families; 14.6% from friends and 41.7% from advertisements.

Table 4: Response of questionnaire administration on usage of indigenous herbal preparations.

Index	Response	% Profile
Preference for Alternative Therapy (Herbal)	Yes	76.3
	No	23.7
Relief from illness after herbal therapy	Yes	64.6
	No	31.4
	Not Indicated	7.6
Perceived potency of herbal therapy	Potent	77.5
	Non-potent	21.5
Reasons for non-preference of herbal medications	Non-prescription of dosages	33.3
	Unhygienic nature	14.6
	Religious beliefs	21.3
	Not Indicated	30.8

Discussion

All the herbal medications used in this study were orally consumed remedies in form of alcoholic spirits, lime, oil, and water-extracted remedies; however, more than 90% of the herbal samples used in this study were water based. In addition, none of the analysed herbal samples had any form of food-based research carried out on them by the manufacturers, which probably accounted for the high recovery rates of coliforms from the herbal medications. Coliforms are members of the family *Enterobacteriaceae* and are the most reliable indicators of faecal pollution, thus the test for their presence is an index of the degree of pollution, which may indicate a possible presence of harmful, disease-causing organisms (APHA 1992; Pelczar et al. 1996; Jay 1997). These bacteria make up approximately 10% of the intestinal microorganisms of humans and other animals and have therefore found widespread use as indicator organisms. Although many pathogens can be detected directly, environmental microbiologists have generally used indicator organisms as an index of possible water contamination by human pathogens (APHA 1992). The significance of faecal coliforms is that if these specific bacteria are present then other harmful microorganisms may also be present, such as *Salmonella* (Forest 2004; Hester 2004) and many more others, which could not be isolated due to the non-availability of appropriate culture media.

The identification characteristics of the isolated pathogens in this study is in agreement with previous works on characterization by Bailey and Scott (1974), Buchnan and Gibbons (1974), Cowan and Steel (1974), Harrigan and McCance (1976), Andrews (1985), Kloos and Schleifer (1975), Sneath et al. (1986), LeMinor and Popoff (1987), Wauters et al. (1988), Aguirre et al. (1990), Bisset et al. (1990), Rodrigues and Kroll (1990), Varnam and Evans (1991), Mossel et al. (1995), Brooks et al. (1998) Rowe-Taitt et al. (2004) and Prescott et al. (2005). All the named pathogens isolated from the indigenous herbal samples in this study have been implicated in previous studies on gastroenteritis and other transmissible diseases (CDC 2002ab; Okeke and Nataro 2001, Ogunshe 2004). *Bacillus cereus* is a bacterium known to cause gastrointestinal infection, which is characterized by diarrhoea (Granum and Lund 1997; McKillip 2000; Phelps and McKillip 2002), while *Staphylococcus aureus* was implicated in gastrointestinal illness by earlier findings of workers such as Sears and Kaper (1996) and Brooks et al. (1998). 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 (Thoerner et al. 2003).

The genus *Salmonella* has been reported to be mostly associated with juvenile gastroenteritis in many countries (Tauxe 1991), while Prescott et al. (2005) reported that *Salmonella* gastroenteritis (salmonellosis) is caused by over 2,000 *Salmonella* serovars. Burnens et al. (1996) and Lindsay (1997) also observed that yersiniosis, caused by *Y. enterocolitica* is known to frequently occur in young children, as enterocolitis with fever, diarrhoea, and abdominal cramps, and this may be due to the fact that *Y. enterocolitica* is known to comprise of strains with different degrees of pathogenicity as earlier reported by Simon et al. (1990) and Thoerner et al. (2003). Chern et al. (2004) also reported that *E. coli* plays a role as diarrhogenic pathogens in infants, and even, according to Liu et al. (2003), the frequency of *E. coli* infection has led to concern over a 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*, *Proteus* and *Vibrio* (Salyers and Whitt 1994) isolated from herbal samples in this study have also been previously reported by Back et al. (1980) and Jiva et al. (1988) in infantile gastroenteritis. *Pseudomonas aeruginosa* was similarly implicated in infantile gastroenteritis transmitted through water and foods by Thom et al. (1970). 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 Guarino et al. (1987). *Proteus mirabilis* and *Morganella morganii* (*Proteus morganii*) have both been reported as well to be associated with diarrhoeal disease by Back et al. (1980).

Nigeria is a country where presently, self-medication is a common phenomenon, since anyone can obtain any form of medication over the counter without a prescription. This is confirmed by the findings of this study in which 79.9 % of the respondents indulge in self-medication. The results in this study clearly show that the popularity of herbal medications among Nigerians is on the increase. Meanwhile pathogenic bacteria were isolated from a total of 237 of the herbal samples analysed in this study without any introduction of contamination during the sampling and testing processes in the laboratory. Most of the herbal medicines analysed in this study would be consumed at room temperature and do not get heated to above 60°C before consumption, thereby increasing the risk of food-borne infections.

In the 1970s, when herbs began their rise in popularity, numerous articles appearing in medical journals and the lay press questioned the safety of herbal products, however, since then, herb usage has increased dramatically, while toxicity reports have not. Perhaps even more important than whether an herbal remedy works, i.e., has the desired therapeutic utility, is the matter of whether it is safe (Tyler et al., 1996; Klein-Schwartz & Isetts, 1996; Hoffman, 1994; Blumenthal et al., 1998). However, the most prevalent difficult situations encountered by majority of the Nigerian patients visiting most of the government-owned or private hospitals have led to the increase in patronage of herbal practitioners for herbal medications as alternative therapy in clinical situations; despite the fact that there are no specific or general critical control or safety standards for the herbal preparations. A large disadvantage of phytotherapy in Nigeria is lack of National Agency for Food and Drug Administration and Control (NAFDAC) oversight as well as consumer indifference to the fact that many herbal preparations contain pathogenic microorganisms.

Although no journal literature could be found on microbial characteristics of indigenous herbal concoctions/medicines in Nigeria, a local report by Kolajo (2000) showed that similar bacterial flora were isolated from some herbal samples with very high recovery rates of pathogenic/clinically-associated microorganisms from the screened herbal preparations as observed in this study. This finding signifies the fact that most of the Nigerian orally consumed herbal medications harbour hazardous microorganisms; meanwhile, more direct/indirect publicity is being given to herbal therapy in the country. This is also supported by the fact that 83.4% of the respondents suggested that government should compliment orthodox medicine with herbal medicine.

It is not unusual to hear of people suffering severe reactions because of overdoses of certain herbs. In some countries there are little or no standards regulating either herbs or practitioners of traditional medicine and this has created the opportunity for herbal fraud and even the sale of dangerous herbal

concoctions passed off as cures (Awake 2000). Food borne illness results from eating food contaminated with bacteria (or their toxins) or other pathogens such as parasites or viruses. The illnesses range from upset stomach to more serious symptoms, including diarrhoea, fever, vomiting, abdominal cramps, and dehydration. Although most food borne infections are undiagnosed and unreported, the Centers for Disease Control and Prevention estimates that every year about 76 million people in the United States become ill from pathogens in food. Of these, about 5,000 die. (NDDICH 2005). In 2004, various outbreaks of gastrointestinal infections were reported in Australia after consumption of scombroid fish at a hotel, redfin fresh water fish caught in a local lake, meal at a graduation dinner and among a group of residents of an aged care hostel in rural Victoria, as recorded in the Victorian Infectious Diseases Bulletin. The presence of large number of coliforms and other food indicator bacterial flora in the analysed indigenous herbal medicines in this study may be due to the methods of preparation herbal medicines or the water sources used in preparing the herbal medicines, since coliforms and similar food indicators are mostly associated with water or water-based samples. We live in a microbial world, and there are many opportunities for food to become contaminated as it is produced and prepared, and despite the fact that these pathogens have different pathogenic doses, most are so pathogenic that even at very small doses they still cause infections. For example, the left over meal consumed by an 11-year old boy who had gastrointestinal and neurological symptoms was sent for toxicity testing but the results were inconclusive (Gregory 2005). It is known that pathogenicity is usually dependent on lethal dose, which varies by strain, and not on microbial counts. According to Jay (1997), among the requirements for foods to be of good sanitary quality, they must be shown to be free of hazardous microorganisms or those present should be at a safe level. Therefore, the high recovery rates of these suspected hazardous microorganisms from indigenous orally consumed herbal medications is of great clinical importance. If the USA and other advanced nations report high mortality due to pathogens in food, one may assume that developing countries, such as Nigeria, that have less effective disease surveillance and monitoring, have significantly under-reported morbidity and mortality from on pathogens in food and unwholesome indigenous herbal medications.

It may be concluded from this study that most traditionally prepared herbal medications in Nigeria are likely to be contaminated with a wide variety of potentially pathogenic bacteria, and that there is insufficient quality control in their production and distribution. The issues raised should be considered by medical and paramedical practitioners, the government and the entire citizenry of the nation. Investigations on bacteriostatic and bactericidal effects of biopreservation of the indigenous traditional herbal preparations are currently going on in our laboratories.

References

Aguirre PM, Cachi JB, Foigueira L. (1990) Rapid fluorescence method for screening *Salmonella spp.* from enteric differential agars. Journal of Clinical Microbiology 28:148-149.

APHA. (1992) Standard methods for the examination of water and wastewater, 18th Ed., Table 9225: I, p. 9-66. American Public Health Association, Washington D.C.

Andrews WH. (1985) A review of culture methods and their relation to rapid methods for the detection of *Salmonella* in foods. Food Technology 39:77-82.

Anon. (1986) Consensus development conference statement: Traveller's diarrhoea. Review of Infectious diseases S223-S227.

Awake (2000) Alternative medicine, Watchtower Bible and Tract Society of New York Inc., USA.

Back E, Molby R, Kaijser B. (1980) Enterotoxigenic *E. coli* and other Gram-negative bacteria of infantile diarrhoea surface antigens, haemagglutinis, colonization factor antigen and loss of enteroxigenicity. Journal of Infection and Diseases 142: 318-327.

Bailey WR, Scott EG, (1974) Diagnostic microbiology. The C.V. Mosby Company, Saint Louis, USA.

Bisset ML, Powers C, Abbott SI, Janda JM. (1990) Epidemiological investigations of *Yersinia enterocolitica* and related species sources, frequency and serogroup distribution. Journal of Clinical Microbiology 28: 910-912.

Blumenthal M, Busse WR, Goldberg A, Hall T, Riggins CW, Rister RS. (1998) The complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines. American Botanical Council, Austin, TX.

Brooks GF, Butel JS, Moore SA. (1998) Medical *Microbiology*. 21st edn. Appleton and Lange, Norwalk, CT.

Buchanan RE, Gibbons NE. (1974) Bergey's Manual of Determinative Bacteriology, 8th edition. Williams and Wilkins Co. Baltimore, USA.

Burnens AP, Frey A, Nicolet J. (1996) Association between clinical presentation, biogroups and Virulence attributes of *Yersinia enterocolitica* strains in human diarrhoeal disease. Epidemiology and Infection 116: 27-34.

CDC. (2002a) Coliform bacteria and drinking water. Centers for Disease Control and Prevention, Atlanta. http://www.bfhd.wa.gov/2002.

CDC. (2002b) Summary of notifiable diseases – United States. Morbidity and Mortality Weekly Report 49:1-102.

Chern EC. Tsai Y. Olson BH. (2004) Occurrence of genes associated with enterotoxigenic and enterohemorrhagic *Escherichia coli* in Agricultural Waste Lagoons. Applied and Environmental Microbiology 70:356-362.

Cowan ST, Steel KJ. (1974.) Manual for the Identification of Medical Bacteria, 2nd edn. Cambridge University Press, London.

Cruickshank R, Duguid JP, Marmion BP, Swain RHA. (1975) Medical microbiology, 12th edn. Churchill Livingstone, NY.

Forest J. (2004) Fecal Coliforms. University of Iowa Hygienic Laboratory Manual, Vol. 36, No. 2, p. 4.

Granum PE, Lund T. (1997) *Bacillus cereus* and its food poisoning toxins. FEMS Microbiological Letters 157: 223-228.

Gregory J. (2005) Enteric diseases-outbreaks of gastrointestinal illness. Surveillance report. Victorian Infectious Diseases Bulletin 8:12-14.

Guarino A, Capano G, Malamisura B. (1987) Production of *Escherichia coli* STa-like heat stable enterotoxin by *Citrobacter freundii* isolated from humans. Journal of Clinical Microbiology 25:110-114.

Harrigan WF, McCance ME. (1976) Laboratory Methods in Food Dairy and Microbiology, p. 342. Academic Press, London, New York, San Francisco, USA.

Hester K. (2004) Fecal Coliforms. University of Iowa Hygienic Laboratory Manual, Vol. 36, No. 2, pp. 5-6.

Hoffman E. (1994) The Information Sourcebook of Herbal Medicine: The Crossing Press, pp1-60. Freedom, CA.

Jay JM. (1997) Modern food microbiology, 3rd Ed. , pp.409-435. CBA Publishers Delhi, India.

Jiva SFH, Krovacek K, Wadstom T. (1988) Enterotoxigenic bacteria in food and water from an Ethiopian community. Applied and Environmental Microbiology 41:1010-1019.

Klein-Schwartz W, Isetts BJ. (1996) "Patient Assessment and Consultation" in Handbook of Non-prescription Drugs, 11th Ed., p87. Covington TR (Ed.). American Pharmaceutical Association, Washington, D.C.

Klipstein FA, Engert RA. (1976) Partial purification of *Enterobacter cloacae* heat-stable enterotoxin. Infection and Immunity 13:1307-1314.

Klipstein FA, Engert RA, Short HB. (1977) Relative enterotoxigenicity of coliform bacteria. Journal of Infection and Diseases 136:205-215.

Kloos WE, Schleifer KH. (1975) Isolation and characterization of staphylococci from human skin, II. International Journal of Systemic Bacteriology 25:62-79.

Kolajo TT. (2000) The study of ecology and microbial flora of some locally prepared herbal medicines in Abeokuta. ND Project, Moshood Abiola Polytechnic, Abeokuta, Nigeria.

Langyan NK, Ahuja M. (2005) Estimation of nickel and cobalt in herbal products, p 220. In: 141st British Pharmaceutical Conference Science proceedings.

LeMinor L, Popoff MY. (1987) Designation of *Salmonella enterica* sp. Nov., nom. Rev., as the type and only species of the genus *Salmonella*. International Journal of Systemic Bacteriology 37:465-468.

Lindsay JA. (1997) Chronic sequelae of food borne disease. Emerging Infectious Diseases 3:443-452.

Liu Z, Lu Y, Zhang J, Pardee K, Wang PG. (2003) P1 trisaccharide (Galα1, 4Gal β1, 4GlcNAC) synthesis by enzyme glycosylation reactions using recombinant *Escherichia coli*. Applied and Environmental Microbiology 69:2110-2115.

McKillip JL. (2000) Prevalence and expression of enterotoxins in *Bacillus cereus* and other *Bacillus* spp., a literature review. Antonie Leeuwenhoek 77:393-399.

Mossel DA, Jansen JT, Struijk CB. (1995) Taking the professional liability for the assurance of the microbiological safety of food and catered meals seriously: preparing for the next millennium by adoption and elaboration of the autonomous total quality strategy, pp 9-29. In: Proceedings of the 4th International Symposium on Microbiology of Food and Cosmetics in Europe. Ispra, Italy, European Common Market Research Center.

NDDICH. (2005) National Digestive Diseases Information Clearing House http://digestive.niddc.nih.gov/index.htm.

Okeke I, Nataro JP. (2001) Enteroaggregative *E. coli*. Lancet: Infectious Diseases 1:304-307.

Okunade AO. (2001) The underdevelopment of health care system in Nigeria, pp.43. Faculty of Clinical Sciences and Dentistry, University of Ibadan. Vantage publishers Ltd. Ibadan, Nigeria.

Ogunshe AAO. (2004) Characterization and selection of *Lactobacillus* species as probiotics for the control of infantile bacterial gastroenteritis. PhD Thesis, University of Ibadan, Nigeria.

Pelczar MJ, Chan ECS, Krieg NR. (1996) Microbiology. International edition. Tata McGraw Hill Publishing Company Ltd., New Delhi.

Phelps KJ, Mckillip JL. (2002) Enterotoxin production in natural isolates of *Bacillaceae* outside the *Bacillus cereus* as a cause of abortion in a mare. Current Science 50:458.

Prescott LM, Harley JP, Klein DA. (2005) Microbiology, 6th Ed., pp. 501-502. WCB/McGraw-Hill, USA.

Rodrigues UM, Kroll RG. (1990) Rapid detection of salmonellas in raw meats using a fluorescent antibody-microcolony technique. Journal of Bacteriology 68:213-223.

Rowe-Taitt CR, Shubin YS, Angel R, Ligler FS. (2004) Detection of *Salmonella enterica* serovar *Typhmurium* by using a rapid, array-based immunosensor. Applied and Environmental Microbiology 69:152-158.

Sakazaki R. (1984) Citrobacter. In: Bergey's Manual of Systematic Bacteriology. Vol. 1. Krieg NR and Holt JG (Eds.). Williams and Wilkins, Baltimore and London.

Salyers AA, Whitt DD. (1994) Bacterial Pathogenesis: A molecular approach. ASM Press, Washington DC.

Sears CL, Kaper JB. (1996) Enteric bacterial toxins: Mechanisms of action and linkage to intestinal secretion. Microbiological Reviews 60:167-215.

Simon M, Richard S, Berche P. (1990) Electron microscopic evidence for *in vivo* extracellular localization of *Yersinia pseudo tuberculosis* harbouring the pYV plasmid. Infection and Immunity 58:841-845.

Sneath PHA, Mair NS, Sharpe ME, Hotts JG. (1986) Bergeys Manual of Systematic Bacteriology, Vol. 2. Williams and Wilkins, Baltimore MD.

Tauxe RV. (1991) *Salmonella*: a postmodern pathogen. Journal of Food Protection 54:563-568.

Thoerner P, Kingombe ClB, Bogli-Stuber K, Bissig-Choi B, Wassenar TM, Frey J, Jemmi T. (2003) PCR detection of virulence genes in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* and investigation of virulence gene distribution. Applied and Environmental Microbiology 69:1810-1816.

Thom AR, Cole AP, Watrasiewiez K. (1970) *Pseudomonas aeruginosa* infection in a neonatal nursery, possibly transmitted by a breast-milk pump. Lancet I:560-561.

Tyler VE. (1996) What pharmacists should know about herbal remedies. Journal of the American Pharmaceutical Association NS36:29-37.

Vanderhoof JA. (2001) Probiotics: future directions. Amnals of Journal of Clinical Nutrition 73:1152S-1155S.

Varnam AH, Evans MG. (1991) Food borne pathogens an illustrated text. Wolfe Publishing Ltd. London.

Wauters G, Janssens M, Steigerwalt AG, Benner DJ. (1988) *Yersinia mallaretti* sp nov. and *Yersinia enterocolitica* biogroups 3A and 3B. International Journal of Systematic Bacteriology 38:424-429.

Wilt TJ, Ishani A, Rutks I, MacDonald R. (2000) Phytotherapy for benign prostatic hyperplasia. Public Health Nutrition 3:459–472.