CARRIAGE OF ENTERIC PATHOGENS AMONG STUDENTS OF A TERTIARY INSTITUTION IN LAGOS, NIGERIA.

D. D. MORO^{1*} K. A. AKINSINDE², B. A. IWALOKUN³, A. U. UWAH2 AND O. FAMUREWA⁴.

- 1. Department of Microbiology, Lagos State University, Ojo, Lagos.
- 2. Genetics Division, Nigerian Institute of Medical Research, Yaba, Lagos.
- 3. Department of Biochemistry, Lagos State University, Ojo, Lagos.
- 4. Department of Microbiology, University of Ado-Ekiti, Nigeria.

ABSTRACT

Sporadic outbreaks of typhoid fever is a common occurrence in most Nigerian cities including Lagos. The source of such outbreaks has been a serious problem of health concern, majority of which are from food handlers and carriers. This preliminary study investigated the carrier state of Salmonella spp. and the incidence of other pathogenic enteric bacteria in the stool of students of Yaba College of Technology in response to the alarming cases of typhoid fever in tertiary institutions in Nigeria. The bacterial isolates were identified based on morphological, cultural, and biochemical characteristics and their antibiotic susceptibility pattern was determined using the disc diffusion technique. A total of 372 isolates were recovered from 300 stool samples which revealed 6.7% and 5.3% carrier rates of S typhi and S. paratyphi A respectively. Shigella spp was recovered from 64 (21.3%) E. coli from 60 (20%), Klebsiella spp from 40 (13.3%) and Proteus spp from 14 (14.7%) of the subjects. The bacterial isolates showed high resistance to tetracycline, colistin sulphate and streptomycin (83-94%), moderate resistance to ampicillin, chloramphenicol, cephalothin and trimethoprim-sulphamethoxazole (5-25%) and low resistance to augmentin and amoxycillin (2-4%). However, all the isolates were susceptible to gentamicin, ceftriazone, cefotaxin, norfloxacin and ofloxacin. Bacterial isolates from these individuals exhibited low resistance to most antibiotics tested suggesting their therapeutic efficacy particularly the third generation cephalosporins and 4-fluoroquinolones in this study. Further studies on mole molecukar epidemiolgy of these isolates would thus provide conclusive evidence.

INTRODUCTION

Food and water borne diseases are serious health problems in both developed and developing countries (Abdusalam, 1984). The situation is rather complicated in countries like Nigeria where there is inadequate health institutions, lack of manpower, portable water with poor preparation and service of food. (Onyemelukwe and Ibe 1993).

The high incidence of typhoid fever reported particularly in Nigeria are often attributed to sporadic outbreaks, the source of which is often difficult to trace (Famurewa and Moro, 1989). Moro et al (2000) reported a high carrier rate S. typhi of (11%) among food handlers who equally harboured other enteropathogens as well as parasitic intestinal worms. These diseases often cause dramatic outbreaks of food poisoning or sporadic epidemics which result in sereve illnesses and death with its huge economic losses.

Food and water borne infections are usually caused by enteropathogens such as viruses, bacteria and parasitic agents (Onyemelukwe and Ibe, 1993) Sour <u>et al</u> (1980) reported that the main disease causing agents were hepatitis A virus, anaecobic bacteria like <u>Clostridium</u> perfringens, enteric bacteria like <u>Shigella</u>, <u>Salmonella</u>, <u>Escherichia coli</u> and parasitic agents like <u>Entamoeba histolytica</u> and <u>Trichinella spiralis</u>, most of which have been reported in other studies by Phonboon <u>et al</u>; 1987 and Famurewa and Moro, 1989.

Transmission of food and water-borne infection is usually by faeco-oral route. Perhaps the most difficult problem confronting our health-care systems is the source of such infection which are on the increase (Moro et al; 2000). Apparently healthy individuals who are carriers are particularly important consequent on their potentials to transmit the disease to either their household or to their customers who constitute the larger society (Famurewa & Moro,

1989). The carrier status can persist for long period of time and is characterized by intermittent and/or continuous excretion of the pathogen (Mims et al; 1998). While food handlers are examined medically before employment, the self-employed food handlers are hardly examined (Moro et al; 2000). Other carriers like students who are not likely to be examined while the pathogen is spread freely as they are involved in preparation and service of food during social gatherings.

Cases of outbreak of typhoid fever in Nigeria is very common on pages of newspapers. The situation is at an alarming proportion as it is a good source of income for laboratory scientists and medical practitioners during diagnosis and treatment respectively, Transfer of pathogenic microorganisms by apparently healthy individuals who harbour them in their bodies constitutes an important problem in the control of several infectious diseases especially of the intestinal tract. However, no effort is made to trace the source(s) of such sporadic outbreaks which often result in high case of fatality. The loss of our leaders of tomorrow to typhoid fever and other food and water-borne infections in recent times in our tertiary institutions prompted this study. We undertook this study to investigate the carrier status of <u>Salmonella</u> and other pathogenic enteric bacteria among undergraduates in Yaba College of Technology and assessed their antibiotic susceptibility pattern

MATERIALS AND METHODS

Sampling Procedure

The study was carried at the Yaba College of Technology, Yaba, Lagos. A total of 300 stool specimens were used for this study which were collected randomly from apparently healthy students. The subjects were enlightened on the purpose of the study having sought their consent and co-operation. The stool samples were then collected from individual student by standard microbiological methods in sterile MacCartney bottles containing buffered peptone water (BPW) in a ratio of 1.10 (w/v) and incubated for 18-24h at 37°C (Cowan, 1993).

Bacteriological Studies

Samples were plated from BPW directly onto MacConkey agar (MAC), Eosin methylene blue agar (EMB), Brilliant green deoxycholate agar (BGDA) Salmonella – Shigella agar (SA) and xylose lysine deoxycholate agar (XLDA) respectively. For the isolation of <u>Salmonella</u>, samples were pre-enriched in BPW for about 18 h followed by enrichment in Rappaport-Vassiliadis medium at 43°C for 18h (Cowan, 1993) Biochemical and serological typing of <u>Salmonella</u> were done according to the method of Edward and Ewing, 1972.

Antimicrobial susceptibility testing

Sensitivity of isolates to antimicrobial agents was determined on Mueller-Hinton agar, using the disc diffusion technique of Finegold et al; 1978. Five colonies of each isolated were emulsified in MacCartney bottle containing 3 ml of normal saline. A sterile cotton swab was then dipped into the suspension and the swab turned against the side of the bottle to remove excess fluid. The inoculated swab was then streaked across the surface of the Mueller-Hinton agar. The inoculated plates were incubated to dry for about 30 mins at 37oC before the antibiotic discs (Abdiscs Abtek Biological Ltd, Liverpool, England) were aseptically placed on them. The plates were incubated at 37oC overnight and the zones of growth inhibition determined. Interpretation of results was done using zone-size interpretation according to Finegold et al: 1978 Antibiotic discs with the following concentrations were used: Ampicillin (10mcg)., cotrimoxazole (25mcg), Chloramphenicol (30mcg), amoxicillin (25mcg), streptomycin (25mcg), tetracycline (25mcg), colistin suphate (25msg), cephalothin (30mcg), augmentin (30mcg), gentamicin (10mcg), ceftriazone (30mcg) cefotaxime (30mcg), norfloxacin (5mcg) and ofloxacin (5mcg).

RESULT

Of the 300 apparently healthy students of Yaba College of Technology, several enteric bacterial pathongens were recovered. Typhoidal <u>Salmonella</u> were recovered from 36(12%), <u>E coli</u> from 60 (20%), <u>Klebsiella</u> spp 40(13.3%) and <u>Proteus</u> spp from 14 (14.7%) (Table 1). A carrier rate of 6.7% and 5.3% was observed of <u>S. typhi</u> and <u>S paratyphi</u> receptively. Table II shows the susceptibility patterns of other bacteria isolated to some commonly used

antimicrobial agents. All isolates showed multiple resistance to antibiotics which included the commonly used ones like amplicillin, tetracycline colistin sulphate streptorrycin, chlorophenicol and cotrimoxanole.

All isolates tested were susceptible to augument, amoxicillin gentamicin ceftriazone cefotaxine, norfloxacin and ofloxacin (Table II.)

Table 1: Bacterial Isolates from 300 apparently healthy students.

<u>Organism</u>	Number positive	Percentage Positive
Salmonella	20	6.7
Salmonella paratyphi A	16	5.3
Shigella spp	64	21.3
E. coli	60	20
Klebsiella spp	40	13.3
Proteus spp	. 14	4

DISCUSSION

Disease outbreaks of one kind or the other are common reports in Nigerian Newspapers especially in our cities. However, these outbreaks are usually not investigated, so the role of food handlers and other asymptomatic carriers in such outbreaks are unknown. Current reports on the incidence of typhoid fever particularly on our campuses are alarming with its attendant mortality rate. According to Sours et al. (1980) in America, the personal hygiene of food handlers was considered a primary contributory factor in outbreaks of food and water-borne infections particularly shigellosis and hepatitis. Onyemelukwe and Ibe (1993) reported the provenance of several enteropathogens in food handlers which they suggested may not differ from what may be seen in the general population. The high incidence enteric bacteria observed in this study confirms that both food handlers and the general population are the likely sources of food and waterborne infections. This emphasizes the need to carry out the overall assessment of all Nigerians. The high incidence typhoidal Salmonella of about 12% from undergraduates in this study is higher even than 11% carrier state carlier reported by Moro et al 2000 among food vendors in Ajegunle, Lagos, thus calls for serious health concern. Similar reports on the carrier rate of both typhoidal and non-typhoidal Salmonella have been reported in Nigeria and elsewhere (Osibo et al; 1975, Phonboon et al; 1987, Famurewa and Moro, 1989; Onyemelukwe and Ibe, 1993, Moro et al; 2000).

Shigella spp was the most common bacterial pathogen identified in the study with an incidence of 21.3%, although they were not serotyphoid. This disagrees with previous studies particularly on food handlers by Famurewa and Moro,. 1989. Moro et al (2000) isolated no Shigella which disagrees with Onyemelukwe and Ibe (1993) who reported that Shigella spp have been incriminated in many disease outbreak and that Shigella flexneraii was the most commonly incriminated (Donald et al, 1987; Panigrahi et al, 1987). Shigellosis may likely be common among the students.

It was observed from the study that all enteric bacterial pathogens isolated showed multiple resistance against most of the commonly used antibiotics. This is likely due to previous exposure to these drugs which are relatively cheaper and readily available. Other factors like self medication, indiscriminate use of incomplete doses of antibiotics usually without prescription have contributed to the emergence of multiple resistance in an alacming proportion. This finding agrees with that of Ogunsola et al; 1997. An urgent need for a good antibiotic policy to guide the therapy of infections diseases is therefore advocated.

The high an-vitro efficacy observed among all the isolates against the less common and expensive antibiotics is quite interesting as it is likely that they have not been abused now. In order to prevent them from being abused they should be protected so that they can only be sold after medication.

ACKNOWLEDGEMENTS

The authors wish to thank the Senate and the Central Research Committee, Lagos State University, Ojo, Lagos for financial support.

REFERENCES

- 1. Abdusalam, M. The role of Food safety in health and development. W. H. O. Chronicle 1984; 38:99-103.
- Bryan, J. P., Rocha, H, Schield W. M. Problems in Salmonellosis; R Rationale for Clinical trials with never Blactam agents and quinolones. <u>Rev. Inf. Dis. 1985; 8.</u>
- Cowan, S. T. Cowan and Steel's manual for the identification of medical bacteria. Cambridge University Press, London 1993, pp 54-60.
- Donald, P. R., Pretorius M. L. and Burger P. J. Shigellosis in the South Western Cape of Good Hope 1968-85
 Epidemiol infect. 1987; 98165-170.
- Edward, R. R. and Ewing, W. H. Identification of Enterobacteriaceac. Burgess Publishing Co. Minesota, USA 1972.
- Famurewa O. and Moro, Incidence of <u>Salmonella</u> and intestinal worms in food handler in Ado-Ekiti, Ondo State Nigeria <u>Acta Mediteranes di pathol. Infett Tropic.</u> 1989, 8; 5-9.
- Mims C. A. Playfair J.H.L, Roitt I. M. Wakelin D, William R. and Anderson R. M. Medical Microbiology Mosby Europe Ltd, 1998; pp. 23.2 – 23.8
- 8. Moro, D. D. Salu, O. B. Oluduro, O. A, Akinsinde, K. A. and O Famurewa. The prevalence of bacterial pathogens and parasitic intestinal worm among food Vendors Ajegunle Lagos. <u>J. Bio & Phys.</u> Sci 2000 (In press).
- Onyemelekwe N. F. and Ibe B. C. Enter-pathrogens in food handlers in Enugu, Nigeria. <u>Nig. Med. pract.</u> 1993; 25(6): 87-89.
- Osibo A. S. Statistics of enteric Gram-negative micro-organisms causing gastrointestinal disorders in Ibadan Nig. J. Med Lab Sci 1975; 2:28-30.
- Panigrahi, D, Agarwal K. C., Venna, A. D. and Bubey M. Incidence of shigellosis and multi-drug resistant shigellae: A 10-year study. <u>J Trop. Med. & Hyg</u> 1987; 90:25-29.
- Phonboon, K, Kunasol, P., Jayaniyothin T and Srisomporn, D. Surveillance of Food and waterborne diseases in Thailand <u>J. Trop Med & Hyg.</u> 1987; 90:311-317.
- Sours, H. E. and Smith D. G. Outbreaks of Foodborne Diseases in the United States: 1972-1978 J. inf. Dis 1980; 122-125.