ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2011 ISBN 1595-689X VOL 12 No.2
AJCEM/200704/2801 *http://www.ajol.info/journals/ajcem*

AFR. J. CLN. EXPER. MICROBIOL. 12(2):58-61

MULTIDRUG RESISTANT *SALMONELLAE* ISOLATED FROM BLOOD CULTURE SAMPLES OF SUSPECTED TYPHOID PATIENTS IN WARRI, NIGERIA.

Ehwarieme,D.A. *Department of Microbiology, Delta State University, Abraka. Abraka, Delta State, Nigeria.*
ayoboladaniel@yahoo.com.

ABSTRACT
This study investigates the prevalence of R-plasmids in *Salmonella sp.* isolated from blood samples of suspected typhoid patients in Warri, Nigeria. A total of 136 blood samples were collected between May and December,2009 and screened for the presence of *Salmonellae* using standard blood culture techniques of which 20(14.7%) was positive for the pathogen. The multidrug resistant (MDR) isolates obtained (n=16; 80.0%), exhibiting the Ampicillin, Chloramphenicol, Cotrimoxazole and Tetracyclin (ACCoT) resistance profile, were subjected to plasmid curing. All (100%) of these MDR isolates bore their resistance markers on plasmids, as they lost their resistance sequel to the curing experiment. The low prevalence (14.7%) of the pathogen in the blood samples indicate that a good number of the suspected typhoid cases may not be incidences of the disease afterall. Furthermore, the high prevalence of MDR and plasmid-mediated MDR (80.0% and 100% respectively) isolates, suggest that treatment failures may be rampant if precise susceptibility test is not conducted prior to prescription.
Key words: Multidrug resistant, blood culture, typhoid fever.

INTRODUCTION

Cases of typhoid fever has become an endemic public health menace especially in developing countries. It is reported that yearly, there are about 16.6million cases of the infection with about 600,000 cases resulting in death (1,2). With the near absence of properly treated public water supply, these alarming figures are expected since typhoid fever is largely spread by the consumption of contaminated water (3). The etiological agent, *Salmonella typhi* is an enteric pathogen and so, the fecal-oral route of transmission is highly significant.

Symptoms of typhoid fever resembles those of some other infections, making diagnosis very paramount in the management of the disease. Routinely, the screening test for typhoid is the Widal serological technique (4) but, the reliability of this test is doubtful. False positive test is commonly encountered (5). Culture therefore remains a more definite diagnostic tool for confirmation of typhoid cases. Stool culture is preferable at about the third week of disease onset, a time when the patient may have been exposed to antibiotic therapy, interfering with chances of recovering the pathogen in the sample. Since there is a 70-90% chances of finding the organism in blood during the first ten days of infection (6,7), the blood culture technique was adopted in this study.

The first line of drugs for treatment of typhoid fever is Chloramphenicol, Amoxicillin and Trimethoprim-Sulfamethosazole (WHO,2003). However, there has been growing concern about the prevalence of Multidrug Resistant (MDR) *S.typhi* in developing nations, Nigeria inclusive (8,9,10). Most of the MDR *S.typhi* indicates plasmid mediation to be of importance. Resistance to the routinely prescribed antibiotics: ampicillin(A), chloramphenicol(C), cotrimoxazole(Co) and tetracycline(T) is usually plasmid- mediated and has been reported widespread from most parts of the world (11,12,13,4).

The oil city of Warri is a classical case of urbanization without development(14). Numerous factors still predispose individuals to typhoid fever infection. This study was designed to obtain the etiological agent of typhoid fever from blood of suspected typhoid patients, determine their pattern of resistance to the commonly prescribed drugs and, make a preliminary enquiry into the involvement of plasmids in the resistance attributes of *S.typhi* isolated from patients in Warri, Nigeria.

METHODS

Collection Of Samples

A total of 136 blood samples were obtained between May and December, 2009 from adults attending various private clinics in Warri, Nigeria, suspected of having typhoid fever, and processed according to standard procedures(7). These were patients with Physician's request for Widal test. Consent of the patients were sought prior to collection of samples. Using aseptic techniques, a sterile syringe and needle was used to withdraw 10mL of venous blood from the patients. Samples were collected twice and inoculated in duplicate into 50mL of Glucose and Sodium taurocholate broth. Incubation was at 37^0C for up till 7 days. Subculture was made onto

MacConkey and Blood agar plates, from broth showing signs of bacterial growth. Initial bacteriological analyses were undertaken at the Microbiology Laboratory of the Delta State University, Abraka. Subsequent plasmid analysis was carried out at the Biotechnology Laboratory of the Lahor Research Labs., Benin-City Nigeria.

Bacterial Identification And Standardization

Bacteria isolates were characterized and identified using standard techniques(15) as previously described (16,17). Stock of isolates were prepared by suspending a loop full of each microbial growth in about 10mL Nutrient Broth(NB). After incubation at 37^0C for 12H, the turbidity was adjusted to be visually comparable with a 0.5 McFarland's standard.

Antibiotic Sensitivity Testing

Sensitivity of the pure culture of bacteria isolates to different antibiotics, was determined using the Kirby-Bauer disc diffusion technique and interpreted based on the guidelines of the Clinical and Laboratory Standards (18). Discs used contained the following antibacterial agents: Ampicillin A(10µg), Chloramphenicol C(30µg), Cotrimoxazole Co(25µg), Tetracyclin T(30µg), Cpx(10µg).

Muella-Hinton (MH) agar plates were swabbed with cells from the bacteria stock solution, already adjusted to the 0.5 MacFarland's turbidity standard. The discs were thereafter, carefully layered on the agar and incubated at 37^0C for 24H.

Plasmid Curing Experiment

All bacteria isolates exhibiting the ACCoT antibiotics resistance pattern, were subjected to plasmid curing experiment (19). 10^3-10^4 cells was inoculated into a series of tubes containing acridine orange at graded concentrations. Incubation was at 37^0C for 24-48H. Subcultures were made from the sub-lethal concentration tube and, again screened for antibiotics resistance. Organisms that lost their resistance after curing were considered to bear the specific resistance marker on plasmids.

RESULTS

A total of 136 blood samples were collected and screened for bacteremia, from suspected typhoid fever patients. Of this number, 111(81.62%) were negative, as no growth was observed in the blood culture after a 7day incubation. However, positive blood culture revealed the occurrence of 20(14.7%), 4(2.94%) and 1(0.74%) respectively for *Salmonella sp., Klebsiella pneumonia* and *Staphylococcus aureus* (Table1).

The resistance profile of *Salmonella* (n=20) isolated, was tested to the antibiotics ampicillin(A=10µg), chloramphenicol(C=30µg), cotrimoxazole(Co=25µg), tetracycline(T=30µg), Nalidixic acid(Na=30µg), gentamycin(G=10µg), amoxicillin(Ax=30µg) and ciprofloxacin(Cpx=10µg). The least resistance was to Cpx,1 (5.0%) and Na,2 (10.0%) while the most of *Salmonella sp.* isolated was resistant to C,18 (90.0%) (Table 2).

Moreover, 16(80.0%) of the 20 isolates exhibited the ACCoT resistance profile. All but CS-003, 004, 006, 009, 010, 014 and 018, were equally resistant to at least one other antibiotic. (Table3).

TABLE1: PERCENTAGE PREVALENCE OF ORGANISMS IN BLOOD OF SUSPECTED TYPHOID PATIENTS.

Organisms	n(%) N=136
Salmonella sp	20(14.7)
Klebsiella pneumonia	4(2.9)
Staphylococcus aureus	1(0.7)

TABLE2: PERCENTAGE RESISTANCE OF *SALMONELLA* TO TEST ANTIBIOTICS.

Drug(concentration)	Resistance n(%) N=20
Ampicillin(10µg)	17(85.0)
Chloramphenicol(30µg)	18(90.0)
Cotrimoxazole(25µg)	16(80.0)
Tetracyclin(30µg)	17(85.0)
Nalidixic acid(30µg)	2(10.0)
Gentamicin(10µg)	10(50.0)
Amoxicillin(30µg)	2(10.0)
Ciprofloxacin(10µg)	1(5.0)

TABLE3: RESISTANCE PROFILE OF *SALMONELLA* ISOLATES BEFORE AND AFTER PLASMID CURING.

Isolates Code number	Before Curing	After Curing
CS-002	ACCoTG	G
CS-003	ACCoT	-
CS-004	ACCoT	-
CS-006	ACCoT	-
CS-007	ACCoTG	G
CS-008	ACCoTG	G
CS-009	ACCoT	-
CS-010	ACCoT	-
CS-011	ACCoTG	-
CS-013	ACCoTG	G
CS-014	ACCoT	-
CS-015	ACCoTG	-
CS-016	ACCoTAx	-
CS-017	ACCoTNaCpx	-
CS-018	ACCoT	-
CS-020	ACCoTNa	Na

DISCUSSION

The percentage of bacterial isolation among patients with typhoid fever vary enormously (20,21) Detection of bacteria by blood culture may be influenced by the culture medium employed, the number of circulating bacteria, the time of blood collection, the volume of blood employed for the culture, the host's immune response system as well as the intracellular character of the bacteria (20,21,19). In the present study, we obtained positive blood culture results from 25(18..38%) of the patients under study.

The low prevalence (14.7%) of the pathogen *Salmonella*, coupled with the presence of other bacteria- *Klebsiella pneumonia* 4(2.94%) and *Staphylococcus aureus* 1(0.74%) in the blood of suspected typhoid fever patients, indicate that some of the patients were not actually suffering from typhoid fever. A good number of infections may present symptoms which are similar to those of typhoid fever. Hence, these patients may not respond to treatment if they are placed on a regime strictly for typhoid fever.

The high prevalence of MDR *Salmonellae* is remarkable. Report from various parts of the world also tend to correlate present findings (22). This goes to suggest that most of the patients will not respond to treatment if placed on these first-line drugs. Equally remarkable, is the fact that all MDR isolates exhibiting the ACCoT resistance pattern lost their resistance sequel to plasmid curing.(Table 3). This is a major clinical challenge since the ease with which resistance markers are transferred, increases with the resistance being plasmid-borne (23).

Of particular interest is isolate CS-017 (Table 3) that equally lost its resistance to the fluoroquinolones(FQs) Na and Cpx after acridine orange treatment. This is a case of an emerging Plasmid Mediated Quinolone Resistance (PMQR). Further research into this isolate is ongoing, to establish whether its FQ resistance is due to a qnr, aac(6')Ib-cr or qep protein which has been established to confer PMQR to certain Enterobacteriaceae (23).

With the rising incidence of typhoid fever infection in our locality, the scenario cannot be any worse, if treatment failure are increasingly encountered. It is thus recommended that proper diagnosis be carried out before establishing cases of typhoid infection. Furthermore, sensitivity tests should inform the choice of drug to be administered.

ACKNOWLEDGEMENT

The assistance of Mr.Bright Igere of Lahor Research Laboratories, Benin-City Nigeria is greatly appreciated.

REFERENCES

1. Ivanoff, B. Typhoid fever: global situation and WHO recommendations. *Southeast Asian J Trop Med Public Health.*(1995) **26**(2): 1-6.
2. Pang,T., Levine,M.M., Ivanoff,B., Wang,J. and Finlay,B.B. Typhoid fever: important issues still remain. *Trends Microbial.,*(1998) **6**:131-133.
3. Rowe,B., Ward,L.R. and Threlfall,E.J. Spread of multiresistant *Salmonella typhi. Lancet* (1990) **337:**1065.
4. Widal,F. Serodiagnostic de la feivre typhoid. *Bull.Mem.Hop.Paris,*(1996) **14**:561-566.
5. Olopoenia,L.A. and King,A.L. Widal agglutination test-100 years later: still plagued by controversy. *Postgrad.Med.J.*(2000),**76**:80-84.
6. Cheesbrough,M. *District Laboratory Practice in Tropical Countries.*Part2. Sheck Wah Tong, Hong Kong.(2004) 434p.
7. Gaviria-Ruiz, M. M. and Cardona-Castro, M.N. Evaluation and comparison of different blood culture techniques for bacteriological isolation of *Salmonella typhi* and *Brucella abortus. Journal of Clinical Microbiology,*(1995), **33**(4): 868- 871.
8. Jesudason, M. V., John, R. and John, T. J. Concurrent prevalence of chloramphenicol-sensitive and multidrug- resistant *salmonella typhi* in Vello, South India. *Epidemiol Infect.*(1996) **116**: 225-227.
9. Kalu,G.I., Ogbulie,E.T. and Opara,F.N. Pattern of multidrug resistant *Salmonella enterica* serovar typhi isolates in Nigeria. *African Journal of Biotechnology*(2008) .**7**(21): 3817- 3820.
10. Thrlfall,E.J., Ward,L.R. and Rowe,B. Widespread occurrence of multidrug resistant *Salmonella typhi* in India. *European Journal of Clinical Microbiology and Infetious Disesse,* (1992) **11:**990-993.
11. Goldstein ,F.W., Chumpitaz, J.C and Guevara, J.M. Plasmid mediated resistance to multiple antibiotics in *Salmonella typhi. Journal of Infectious Disease.*(1986) **149**:652
12. Ling,J. and Chau,P.Y. Plasmid multidrug resistance to chloramphenicol, trimethoprim and ampicillin in *Salmonella typhi* in the south-east Asian region. *Journal of Infectious Disease* (1984) **149**:652.
13. Thrlfall,E.J., Ward,L.R. and Rowe,B. Widespread occurrence of multidrug resistant *Salmonella typhi* in India. *European Journal of Clinical Microbiology and Infetious Disesse,* (1992) **11:**990-993.
14. Ejechi E.O. and Ejechi B.O. Sociological dimension in the handling habit and Sanitary quality of hand-dug well water from oil-producing area of Nigeria. *Environmental Monitoring and Assessment*,(2007) 134: 255-261.
15. Cowan, S.T. and Steel, S. *Manual for the Identification of Medical Bacteria.* 3rd edition, Cambridge University Press(1993).

16. Ehwarieme,D.A., Egbule,O.S. and Okonjo,N.P. Antibotic resistance profile of soil-borne enteric bacteria isolated from parts of Delta State, Nigeria. *Journal of Applied Sciences,*(2010), **13**(1):8949-8958.
17. Enabulele,O.I., Ehwarieme,D.A. and Aluyi,H.S.A. Resistance pattern of *Salmonella* isolates from food, animal and human sources, to common antimicrobial agents. *Global Journal of Pure and Applied Sciences,*(2008), **14**(2):179-182.
18. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing*. *17*th International Supplement. CLSI M100- S17(2001). Clinical and laboratory standards Institute, Wayne,P.A.
19. Sullivan,N.M., Sutter,V.L. and Finegold,S.M. Practical aerobic membrane filtration blood culture technique: clinical blood culture trial. *Journal of Clinical Microbiology,*(1975) **1**:37-43.
20. Born, G. L., Haynes, J.R. and Burson, G. G. Blood culture technique on developmental phase. *Journal of Clinical Microbiology*, (1976)**3**: 251- 257.
21. Herlich, M. B., Schell, R.F., Francisco,M. and Le Frock,J.. Rapid detection of stimulated bacteremia by centrifugation and filtration. *Journal of Clinical Microbiology.*(1982) **16** : 99-102.
22. Mandal,S., Mandal,M.D. and Pal,N.K. Plasmid encoded multidrug resistance of *Salmonella typhi* and some enteric bacteria in and around Kolkata, India. *Online Journal of Health and Allied Sciences,*(2004) **3**(4):19-24
23. Lazaro,N.S., Tibana,A., Rodrigues,D.P., Reis,E.M.F., Quintaes,B.R. and Hofer,E. Antimicrobial resistance and R-plasmid in *Salmonella spp.* from swine and abattoir environments. *Pesq. Vet. Bras,*(2004) **24**(2):57-60.