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Full Length Research Paper

Detection of Blactx-M gene on resistant *salmonella enterica* from a hospital in southeast Nigeria

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Typhoid fever is said to be a systemic infection caused by *Salmonella Enterica* serotype *typhi*. A total of fifty stool samples were analyzed out of which twenty-five clinical isolates of *Salmonella enterica* serovars recovered between July-September, 2010 were investigated and screened using molecular methods for the presence of Blactx-M type gene. Polymerase Chain Reaction analysis showed that the four of the *S.enterica* encoding the CTX-M type gene were mostly Extended Spectrum β-lactamase producers which had no plasmids DNA. Phenotypic analysis reveals that the enzymes were active on Ceftazidime (64%) more than Cefotaxime (16%). Two folds serial dilution method reveals that Cefotaxime had increased Minimum Inhibitory Concentration (MIC) ranging from 1.56ug/ml- 12.5ug/ml depending on the isolates and the *β*-lactamase enzyme produced. The four Blactx-M genes detected on the adult patients were from the General Out Patients Department (GOPD) and National Health Insurance Scheme (NHIS) section of the hospital in Eastern Nigeria.

Key words: Blactx-M, ESBL, *Salmonela enterica*, PCR, Cefotaxime, Ceftazidime.

INTRODUCTION

The emergence of resistance to antimicrobial agents within the ***Salmonellae*** is a worldwide problem that has been associated with the use of antibiotics in livestock. Resistance to Ceftriaxone and the Fluoroquinolones, which are used to treat invasive ***Salmonella*** infections, is rare in the United States (Fey,et.al., 2000). Typhoid fever is a systemic infection caused by *Salmonella Enterica* serotype *typhi*. This is a highly adapted human specific pathogen and possesses remarkable mechanisms for persistence in host (Mushtaq, 2006). Most of the disease burden occurs in developing countries due to poor sanitary conditions (Parry,et.al.,2002). Cefotaxime is a third-generation **cephalosporin antibiotic, like other third-generation cephalosporin,** it has broad spectrum activity against Gram positive and negative bacteria. In most cases, it is considered to be equivalent to Ceftriaxone in terms of safety and efficacy. Cefotaxime is used for infections of the respiratory tract, skin, bones, joints, urogenital system, meningitis, and septicemia. The *syn*-configuration of the methoxyimino moiety confers stability to ***β*-lactamase** enzymes produced by many **Gram-negative bacteria (**Bonnet, 2004). Studies have also shown that resistance to broad-spectrum *β*-lactams is highly mediated by extended-spectrum ***β*-lactamase** (ESBL) enzymes, increasing the world health problem in clinical settings (Ling, 2006; Yah, et.al., 2007b; Valverde, et.al., 2008). According to Yah, et.al.,(2007b), the plasmid-borne ***β*-lactamases** are also competent enough to hydrolyze ***β*-lactam** antibiotics, as well as the mechanism of resistance to ***β*-lactam** agents among gram-negative bacteria. Bacteria have acquired a variety of mechanisms to resist the action of antibiotics. The production of ***β*-lactamases**, enzymes that destroy penicillin's and cephalosporins by hydrolyzing their ***β***-lactam nucleus, is the most common mechanism of resistance (Jalil, et.al., 2005). Cefotaximases (CTX-M) are class A ***β*-lactamases** that generally present higher levels of hydrolytic activity against Cefotaxime than against Ceftazidime. Ceftazidime MICs for organisms

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susceptible range. Many laboratories use Ceftazidime resistance alone as an indicator of extended-spectrum ***β*-lactamase** production (Batchelor, et. al., 2005). More than 80 CTX-M enzymes are currently known. Despite their names, few are more active on Ceftazidime than Cefotaxime. They have been found mainly in strains of *Salmonella enterica* serovar *Typhimurium* and *E. coli*, but have also been described in other species of Enterobacteriaceae and are the predominant ESBL type in parts of South America (Woodford, et.al., 2006). Thus the use of molecular tool like Polymerase Chain Reaction (PCR) to detect this resistant gene in organism and to stop the spreading of this gene is essential, especially in this part of Africa. Since only phenotypic pattern is not discriminating enough to distinguish this organism, as it depends also on the growth of the organism especially *S. enterica* serotype typhi. This study was aimed at using a fast, sensitive and specific molecular method such as PCR in detecting the prevalence of Blactx-M type gene, and Extended Spectrum *β*-lactamases (ESBL's) in Nigeria, most especially in the Southeast region of Nigeria where antibiotics resistance to Cefotaxime and Ceftazidime are in the increase in the Hospitals.

MATERIAL AND METHODS

Bacteria Isolates;

In a total of 50 stool samples collected and analyzed from the several units in the federal medical centre(FMC) Owerri hospital (July- September,2010), 25 isolates of *S.enterica* serovars producing Beta-lactamase were obtained using microbact identification kit(Oxoid,England). Clinical human isolates of the *Salmonella enterica* used were obtained from the Routine Section of the Medical Microbiology Laboratory FMC in the Southeast part of Nigeria after basic culturing techniques and biochemical characterization. Also used for the analysis is a reference strain negative of Blactx-M type gene *S.enterica serovar typhimurium* ATCC14028.The antibiotic- sensitivity screening was carried out by the multidisc agar diffusion method on 20ml molten Muller Hilton Agar according to (NCCLS,2003) standard using these antibiotics disc(Oxoid, India); Co-trimoxazole, Chloramphenicol , Ciprofloxacin , Amoxicillin ,Streptomycin, Levofloxacin , Ceftriaxone , Cefotaxime , Ceftazidime , Amoxicillin/Clavulanic acid . The tube dilution method of broth was used to determine the MIC of the test antibiotic against the clinical strains by the two fold serial broth dilution procedure according to (NCCLS 2003).

β -lactamase detection using Nitrocefine sticks

Nitrocefin (Oxoid, England) is the chromogenic cephalosporin developed by Glaxo Research Limited coded 87/312; 3-(2, 4 dinitrostyrl) –6R, 7R) –7-(2 thienylacetamido)-ceph-3-em-4 carboxylic acid, E-isomer. The Nitrocefin container was removed from the freezer and allowed to reach room temperature. Then a well separated representative colony from the primary isolation medium was selected. One of the stick (color coded black) was removed from the container and holding the colored end, the colony was touched with the impregnated tip of the stick, which is rotated to pick up a small mass of cells. The inoculated tip of the stick is placed between the lid and the base of the inverted plate. The reaction requires moisture, so the inoculated tip of the stick is placed in the moisture condensate in the lid or if condensate is not available in the inverted lid, one or two drops of distilled water were added to the lid to moisten the tip of the stick. The reagent was examined (impregnated tip of the stick) for up to five minutes and, if negative it was re-examine after fifteen minutes. A positive reaction was shown by the development of a pink/red color. No color change was observed with organisms that do not produce beta-lactamase. To ensure correct reading of the colored nitrocefin stick, it was compared to an unused nitrocefine stick.

Serotyping using Slide Agglutination;

The slide agglutination antigen kit (Lab-Care Diagnostics, India) was used for this analysis. The cultured colony on a nutrient agar medium were isolated and inoculated on the grease free slide. A drop of physiological saline solution was introduced and the contents mixed using a mixing stick. The results were observed macroscopically after one minute, for auto-agglutinating strains after rocking the slide for 30–60 seconds. This procedure was repeated simultaneously with a drop of the antiserum (Lab-Care Diagnostics, India). The O antigen suspensions were tested first. If they were positive, then the monovalent O antisera belonging to this group were also performed. A positive result shows that the isolated bacterium possesses the antigen corresponding to the antiserum. The same procedures were subsequently carried out on the H antigen suspension (Cheesbrough, 2006).

Plasmid Extraction;

The Alkaline Lysis method of (Winstanley and Rapley, 2000; Perez-Roth, et.al., 2001) was adopted for the extraction. 200ul of buffer 1A (400Mm Tris, 20mM Na EDTA, Acetic acid to pH 8.0) was added to the cell pellets and vortexed. Then 400ul of the lysing solution (4% SDS (Sodium Dodocyl Sulphate) was added and the tubes were inverted 20 times at room temperature and 300ul ice cold buffer2B (3M Na Acetate, Acetic acid to pH 5.5) was added. Vortex the mixture and keep on

ice for 30 minutes. Then centrifuge at 3,000xg for 15 mins. To the supernatant 700ul of chloroform was added and vortexed. Centrifuge at 3,000xg for 10 mins. and transfer 500ul of the aqueous layer into a new tube. Then add 1ml of absolute ethanol and keep on ice for 1 hr. Centrifuge at 3,000xg for 30mins. Then wash pellets with 70% ethanol, decant and dry pellets. 50ul of buffer 3C (10Mm Tris, 2Mm Na2 EDTA, Acetic acid to pH 8.0.) was added and the kept for electrophoresis on ice.

Detection of ESBL's Positive Isolates using Double Disc Synergy Test (DDST)

The test inoculums (0.5 McFarland turbidity) were spread onto Mueller-Hinton agar (MHA; Oxoid, India) using a sterile cotton swab. A disc of augmentin (Amoxicillin + Clavunalic acid) was placed on the surface of MHA; then discs of Ceftriaxone (CRO) ,Ceftazidime (CAZ) and Cefotaxime(CTX) were kept around it in such a way that each disc was at distance ranging between 16 and 20 mm from the augmentin disc (centre to centre). The plate was incubated at 37 °C overnight. Distances between the discs were required to be suitably adjusted for each strain in order to accurately detect the synergy. The organisms were considered to be producing ESBL when the zone of inhibition around any of the Broad-spectrum cephalosporin discs showed a clear-cut increase zone of inhibition towards the augmentin disc (Yah, 2007b).

Rapid Extraction, Quantification and Purity Test of the DNA;

1.5ml of an overnight broth culture was pipetted into eppendorf tubes and centrifuged at 5xg. The supernatant was discarded and sterile water was introduced into the tubes and the mixture is shaken vigorously by a vortexing machine and decanted. This process was repeated twice. Then the mixture was resuspended in 100ul sterile distilled water, vortexed and placed in a water block to boil for 10-20mins at 100o C. After boiling, the mixture was then centrifuged for 10 mins and the purified chromosomal DNA pellets were transferred into a new tube and stored on ice for further analysis. The spectrophotometer lens (Nano drop ND1000) was cleaned with sterile distilled water and 0.2ul drop of the DNA sample was added on the lens, closed and then clicked to measure on the computer screen and the readings was observed. Purity level is known to be between 1.5 - 1.8 or 2ul (data not shown).

PCR Amplification and Agarose Gel Electrophoresis;

The purified DNA template in the PCR eppendorf tubes (2.0ul DNA) was mixed with the Master mix ready to load (Solis Biodyne with 7.5mM MgCl2) containing all the required contents as stated by the manufactures descriptions except water and the primers for the amplification. The primers mixtures (Biomers diagnostics), DNA and the Master mix was vortexed to mix and then centrifuged before introducing it into the PCR machine (Eppendorf-Germany).Then the machine is adjusted to annealing temperature and switch to start. After amplification has completed, the PCR products were then taken for electrophoresis (CBS.Scientific Company Inc.) on the agarose gel electrophoretic machine at 80-100volts and finally viewed on the UV light for visible amplified image of the genes. A DNA-marker of 100base pairs was used.

The Polymerase Chain Reaction (PCR) were performed under the following conditions with the (Solis biodyne 5x FIREPol) Master Mix Ready to load. The thermo cycling condition used for Blactx-M were 30 – 35 cycles of 95oC for 30secs, 72Oc for 1min.,66.2 for1 min., 72Oc for 1min, 95oC for 30 sec.,(PCR timing 1.38- 2.58 hrs). The Blactx-M universal primer used were; Blactx-M - F(5'ATG TGC AGY ACC AGT AAR GTK ATG GC-3'), R(5'- TGG GTR AAR TAR GTS ACC AGA AYC AGC GG- 3') where R in the sequence is purine,Y is pyrimidine, and S is G or C (this is to design to occupy for the ambiguity of sequence variation among the CTX-M types).

RESULTS AND DISCUSSION

Typhoid fever is a systemic infection caused by *Salmonella Enterica* serotype *typhi*. This is a highly adapted human specific pathogen and possesses remarkable mechanism for persistence in host (Mushtaq, 2006). Most of the disease burden occurs in developing countries due to poor sanitary conditions (Parry, et.al., 2002). The results of the antibiotics screening using agar diffusion method were positively discovered in this study. Several reports on how to extract DNA rapidly from overnight liquid culture as procedures for DNA extraction directly from a single colony as stated in (Winstanley and Rapley, 2000). In this study we described and adopted a rapid method of DNA extraction directly from a single bacterial colony that gave quality DNA for PCR in 15-20 mins. This protocol also gave quality DNA products for PCR amplification and was tested for its quantity and purity which resulted in quality PCR fragments.

The report of the antibiotics resistant pattern of the twenty-five *S.enterica* isolated from stool as analyzed in this study were as follows ; Amoxicillin/Clavulanic acid (92%), Amoxicillin (76%), Chloramphenicol (68%), Streptomycin (68%), Ceftazidime (64%), Co-trimoxazole (64%), Ciprofloxacin (24%),Cefotaxime (16%), Levofloxacine(12%), Ceftriaxone(12%). The results of the phenotypic expression analysis revealed

Table 2: PHENOTYPIC RESISTANCE PATTERN IN RELATION TO *β*- LACTAMASE AND ESBL'S PRODUCTION IN *S. ENTERICA* SEROVAR.

CODE S/NO	NUMBER OF ANTIBIOTICS	RESISTANCE PHENOTYYPIC PATTERN	*β* -LACTAMASE PRODUCTION	PRESENCE OF ESBL'S
SO3	2	CH, AMC	+VE	ESBL
SO9	7	SXT, CH,CPX,AM,STR,CAZ,AMC	+VE	ESBL
SO10.	6	SXT,CH,CPX,AM,CAZ,AMC	+VE	ESBL
SO11.	8	SXT,CH,CPX,AM,GEN,STR,CAZ,AMC	+VE	ESBL
SO12.	7	SXT,CH,CPX,AM,STR,CAZ,AMC	+VE	ESBL
SO13.	5	SXT,CH,AM,STR,AMC	+VE	ESBL
SO14.	9	SXT,CH,AM,STR,LEV,CRO,CTX,CAZ,AMC	+VE	ESBL
SO15.	5	SXT,CH,AM,STR,AMC	+VE	ESBL
SO19.	6	SXT,CH,CPX,AM,STR,AMC	+VE	ESBL
SO24.	6	AM,STR,CRO,CTX,CAZ,AMC	+VE	ESBL

KEY: **SXT**= Co-trimoxazole, **CH**= Chloramphenicol, **CPX**= Ciprofloxacin, **AM**= Amoxicillin, **STR**= Streptomycin, **LEV**= Levofloxacin, **CRO**= Ceftriaxone, **CTX**= Cefotaxime, **CAZ**= Ceftazidime, **AMC**= Amoxicillin/Clavulanic acid, +**ve** = Positive, **ESBL**= Extended Spectrum Beta Lactamase., SO= ***Salmonella* Owerri**

Table 3: Minimum Inhibitory Concentration (MIC's) of some of the antibiotics tested on the Bla_{ctx-M} positive *S.enterica* serovars

CODE S/NO	ANTIGENIC PROPERTY	LEV (MIC)	CPX (MIC)	AMC (MIC)	CTX (MIC)	CRO (MIC)
SO3	*S.enterica* (O/H)	1.56	3.125	25.0	3.125	1.56
SO14	*S.enterica* (O/H)	25.0	3.125	25.0	6.25	6.25
SO15	*S.enterica* (O/H)	3.125	1.56	25.0	1.56	3.125
SO24	*S.enterica* (O/H)	1.56	1.56	25.0	12.5	12.5

KEY: **SXT**= Co-trimoxazole, **CH**= Chloramphenicol, **CPX**= Ciprofloxacin, **AM**= Amoxicillin, **STR**= Streptomycin, **LEV**= Levofloxacin, **CRO**= Ceftriaxone, **CTX**= Cefotaxime, **CAZ**= Ceftazidime, **AMC**= Amoxicillin/Clavulanic acid, +**ve** = Positive, **ESBL**= Extended Spectrum Beta Lactamase., O = Somatic antigen, H= Flagellated antigen, SO= ***Salmonella* Owerri.**

that the subsequently *β*-lactamase were more active on the Ceftazidime, than on the Cefotaxime as shown (Table 2). This may be in support of the studies carried out by several authors revealing that resistance to broad-spectrum *β*-lactams is highly mediated by extended-spectrum *β*-lactamase (ESBL) enzymes, which has increased the world health problem in clinical settings (Ling, 2006; Yah, et.al., 2007b; Valverde, et.al., 2008). According to Soge, et.al., (2005) the first CTX-M-type *β*-lactamases were identified as plasmid-encoded enzymes in clinical isolates from the Enterobacteriaceae, and all 30 isolates of *Klebsiella pneumonia* in their study produced at least one β-lactamase and 17 (57%) produced a CTX-M *β*-lactamase (Soge, et.al., 2005).

The PCR amplification result in this study shows that a total of four isolates of *S.enterica* were positive for Blactx-M gene type using a universal primer (two male and two female adult patients) (Fig.1), but no plasmids were found encoding the gene of the *S.enterica* from the four patients. This also shows that it is not all *β*-lactamase enzyme that causes resistant to antibiotics are mediated by plasmids, and that there are other mechanism of resistance like Blactx-M gene or Cefotaximases enzymes that mediates drug resistance especially in *S.enterica*, also that among ESBL family, the CTX-M type of enzyme is widely spread in many organisms in the world especially *S.enterica* serovars. Furthermore, the Cefotaxime was seen to have increased MIC'S which ranges from 1.5ug/ml in isolates CODESO3 to isolates CODESO14 with MIC 6.25ug/ml. Other MIC of the Cefotaxime on isolate CODESO15 and CODESO24 were 1.56ug/ml and 12.5ug/ml respectively (Table3). From Table 1, in this study, analysis reveals that both the *S.enterica* isolated from the female and male adults having positive Blactx-M type gene were all found to produce ***β*-lactamase** and were also recovered from the General Out Patients Department (GOPD), except isolate CODESO24 which were isolated from a patient on National Health Insurance Scheme(NHIS). Though all the *S.enterica* positive to Blactx-M gene were resistance to various number of antibiotics, isolates CODESO3 was only resistant to 2 antibiotics, isolate CODESO14 was resistant to Nine of the antibiotics tested, while isolate CODESO15 and CODESO24 were resistant to 5 and 6 antibiotics respectively. Also analysis revealed that out of the 25 *S.enterica* tested, 10(40%) of the isolates

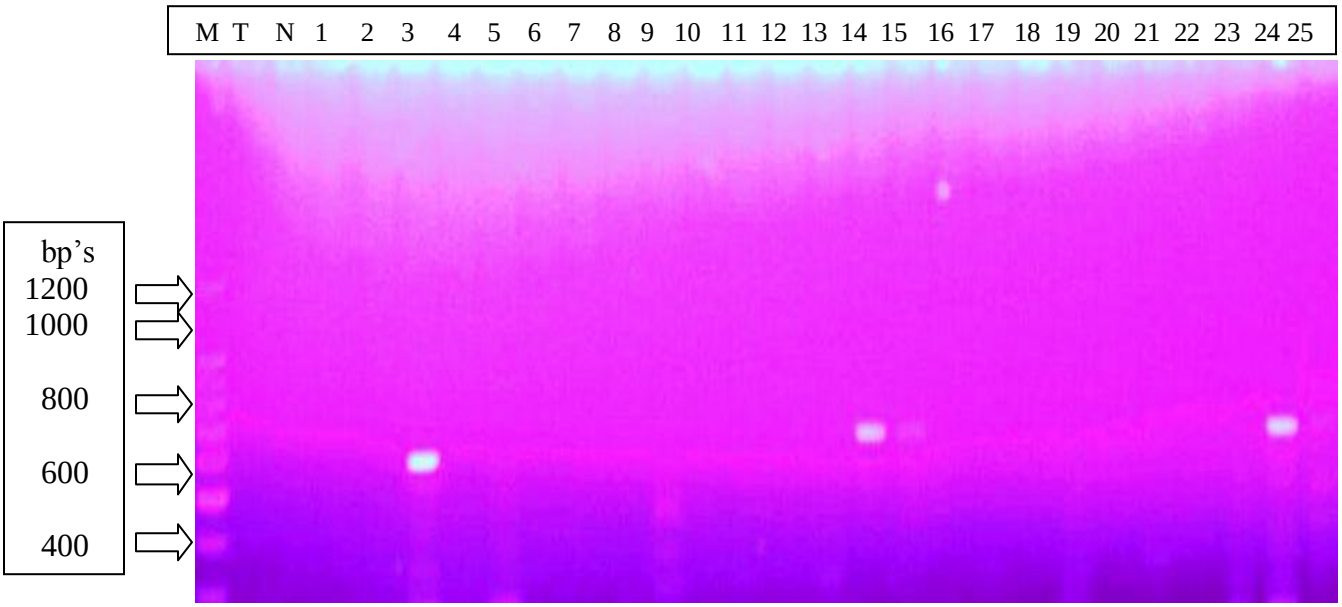

Figure1: Agarose gel electrophoresis pattern showing single PCR amplification products of Bla_{ctx-M} genes

Table 1: EPIDEMIOLOGICAL PATTERN OF DISTRIBUTION OF PATIENCE DATA AND MOLECULAR ANALYSIS OF THE Bla_{ctx-M} TYPE GENE OF *S. ENTERICA* DETECTED FROM THE HOSPITAL IN THE EASTERN PART OF NIGERIA.

Code S/NO	Unit/ Dept.	Sex	Age Range (yrs)	B-lactamase/ CTX-M Production	Presence of Plasmid/Mwt.	No of antibiotics Resistant
SO3.	GOPD	F	Adult (18-above)	+ve/CTX-M	Nil	2
SO14.	GOPD	M	Adult (18-above)	+ve/CTX-M	Nil	9
O15.	GOPD	F	50	+ve/CTX-M	Nil	5
O24.	NHIS	M	Adult (18-above)	+ve/CTX-M	Nil	6

KEY: F= Female, M= Male, NHIS = National Health Insurance Scheme, GOPD= General Out Patience Department .**Note:** Adult (includes male and female between 18 and above, whose actual age was not determined).Nil= Negative, CTX-M = Cefotaxime-M type enzyme, SO= ***Salmonella*** **Owerri**

produced ***β*-lactamase** enzyme while only 8(24%) were ESBL positive. Though not all of the ESBL positive *S.enterica* produced the Blactx-M type gene but all possessed the somatic and flagellated antigenic properties. From the analysis the resistant to Ceftriaxone (12%) were less when compare to Cefotaxime (16%), while the resistance to Ceftazidime (64%) were higher. It should be noted that some organisms will not exhibit lactamase unless the enzyme has been induced by exposure to a beta-lactam antimicrobial and are called chromosomal Cephalosporinase, so some of the isolates which were resistant to some of the cephalosporin's, like Ceftazidime, Ceftriaxone and other combination of Amoxicillin/Clavulanic acid, may be due to this type of enzyme called Cephalosporinase. Although all the inhibitor-resistant ***β*-lactamases are not ESBLs**, they are often discussed with ESBLs because they are also derivatives of the classical TEM- or SHV-type enzymes according to (Bradford, 2001; Soge, et.al., 2005). Also, according to Batchelor, et.al., (2005), Cefotaximases (CTX-M) are class A ***β*-lactamases** that in general present higher levels of hydrolytic activity against cefotaxime than against ceftazidime. But in some cases Ceftazidime MICs for organisms producing these enzymes are sometimes in the susceptible range. Also reports by Batchelor, et.al., (2005) stated that many laboratories use ceftazidime resistance alone as an indicator of extended-spectrum ***β*-lactamase** production. In this study as earlier stated Ceftazidime resistance was high, revealing the presence of this enzyme Phynotypically. Furthermore, analysis reveals that there were Multi drug Resistant (MDR) *S.enterica* based on the resistant number of antibiotics.

CONCLUSION

Many studies have demonstrated the use of molecular methods in the identification of specific bacterial genes that causes resistant in several organism especially, *S.enterica*. In this analysis the ability of PCR and the combination of other molecular method to achieve this purpose has been revealed. The identification of the Blactx-M type gene by PCR which was used to target the gene using primer sequences, has also shown that molecular methods is highly specific. The detection of ESBL's and ***β* -lactamase** as one of the mechanism of resistance used by *S.enterica* in this study has been revealed. Also , in this study, the first line of drugs such as Chloramphenicol, Co-trimoxazole and even the combination of Amoxicillin/ Clavulanic acid used for the treatment of *S.enterica* was also seen to be highly resisted by this organisms due to the presence of this enzymes in the genes . Therefore care should be taken in the way most of these drugs are prescribed and used by patients in the community or in the hospitals. Proper diagnostic analysis is highly required and suggested for this purpose. Further molecular analysis such as DNA sequencing is required to completely identify the actual CTX-M type of enzyme encoding the gene of this highly pathogenic organism.

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REFERENCES

Batchelor M, Hopkins K, Threlfall JF, CliftonHadley A, Stallwood AD, Davies RH, Liebana E (2005). *bla*CTX-M Genes in Clinical *Salmonella* Isolates Recovered from Humans in England and Wales from 1992 to 2003. Antimicrob. Agents Chemother. 49(4):1319-1322,

Bonnet R (2004). Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother. 48:1-14

Bradford PA (2001). Extended-spectrum *β*-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 48:933-51.

Cheesbrough M (2006). District laboratory practices in tropical countries. 2nd ed.(2). Cambridge University, press. U.k.182-187.

Fey PD, Safranek TJ, Rupp ME, Dunne EF, Ribot E, Iwen PC, Bradford PA, Angulo FJ, Hinrichs SH, (2000) Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle. N. Engl. J. Med. 27;342(17):1242-9.

Jalil A, Wadood A, Rehman JU, Naseem R, Hussain A, Ali J (2005) Comparative susceptibilities of *Escherichia coli* to ceftriaxone alone and in combination with sulbactam. Pakistan J. Med. Res. 44(1).

Mushtaq MA (2006) What after ciprofloxacin and ceftriaxone in treatment of Salmonella typhi. Pak. J. Med. Sci . 22 : 1 51-54.

National Committee for Clinical Laboratory Standards (2003). Performance standard for antimicrobial susceptibility testing,13th informational supplements. *Approved standards M100-S13(M2)*NCCLS,Villanosa,PA,USA.

Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ (2002) Typhoid Fever. New Engl. J. Med. 347: 1770-82.

Perez-Roth E, Claverie-Martins E, Villar J, Mendez-Alvarez S (2001)Multiple PCR for Simultenous Identification Staphylococcus aureus, Detection of Methicillin and Mupirocin Resistance. J. Clin. Microbiol. 4037-4041.

Soge OO, Queenan AM, Ojo KK, Adeniyi BA, Roberts MC (2005) CTX-M-15 extended-spectrum β-lactamase from Nigerian Klebsiella pneumonia. J. Antimicrobial Chemotherapy. 57: 24–30.

Valverde A, Grill F, Veresa MC, Pintado V, Fernando B, Rafael C, Cobo J (2008). High rate of intestinal colonization with extended-spectrum-lactamase-producing organisms in household contacts of infected community patients. J. Clin. Microbio. 46(8): 2796-2799.

Winstanley C, Rapley R (2000) The Nucleic Acid Protocols Handbook: Extraction and Purification of Plasmid DN, Humana Press Inc., Totowa, NJ.

Woodford N, Ward E, Kaufmann ME (2006). Molecular characterization of *Escherichia coli* isolates producing CTX-M-15 extended-spectrum *β*-lactamase (ESBL) in the United Kingdom. Health Protection Agency. 11-19.

Yah SC, Chineye HC, Eghafona NO (2007b). Multi antibiotics-resistance plasmid profile of enteric pathogens in pediatric patients from Nigeria. Biokemistri. 19(1): 35-42.

Yujuan J, Ling JM (2006) CTX-M-producing *Salmonella spp.* in Hong Kong: an emerging problem. J. Med. Microbial. 55: 1245-1250.