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Full Length Research Paper

Survey of 3rd generation cephalosporin genes in multi-resistant *Salmonella* serotypes from septic poultry and an asymptomatic healthy pig from Nigeria

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Occurrence and spread of *Salmonella* genes encoding AmpC and extended-spectrum beta-lactamases (ESBL) is a major public health problem worldwide. These genes have been identified in *Salmonella* serotypes all over the world yet there is paucity of reports on these genes in Nigeria, despite the phenotypic evidence of resistance to beta-lactam drugs. The current work used a multiplex PCR to identify beta-lactam resistance genes in five Nigerian-origin *Salmonella* isolates exhibiting resistance to third-generation cephalosporins. The isolates included four strains isolated from septic poultry (two strains of *Salmonella enterica* serotype Kentucky and two strains of presumptive *S. enterica* serotype Pullorum) and one *S. enterica* serotype Give isolated from one of two hundred asymptomatic pigs. The predominant genes found in these Nigeria serotypes include: TEM, SHV, GES, OXA-2, ACCM, FOX, ECBM and DHAM. The presence of these plasmid-borne genes underscores the potential health risk of antibiotic resistance transfer from food animals to human in Nigeria because third-generation cephalosporin drugs are still the drug of choice in treating life-threatening systemic infections in Nigeria.

Key words: *Salmonella*, 3rd generation cephalosporin, multi-resistant, Nigeria.

INTRODUCTION

Non-typhoidal *Salmonella* spp. have been described as major pathogens associated with food- borne gastro-enteritis worldwide (Threlfall, 2002). While antibiotics are not usually recommended in cases of *Salmonella* enterocolitis, their use for therapeutic purpose becomes important when the pathogen becomes invasive as in meningitis, sepsis and bacteraemia (Threlfall, 2002). In cases of such life-threatening complications, fluoro-quinolones and extended-spectrum cephalosporins are usually the drug of choice (Hohmann, 2001). The use of the extended-spectrum cephalosporins are however, particularly recommended when children are involved because of fluoroquinolone-mediated chondrotoxicity in in this group (Hohmann, 2001). Following the occurrence of lactamases (ESBLs) and AmpC-type beta-lactamases, resistance to the extended-spectrum cephalosporins among members of the family *Enterobacteriaceae* has since grown to be a worldwide public health problem (Bradford, 2001). The principal mechanism of resistance to the extended-spectrum beta-lactam antibiotics involves the production of ESBLs and AmpC beta-lactamases in *Enterobacteriaceae* (Shahada et al., 2010). The ESBLs hydrolyze oxyimino-cephalosporins and monobactams, but not cephamycins and they can sometimes be inhibited by clavulanic acid (CVA) (Shahada et al., 2010). The AmpC beta–lactamases on the other hand hydrolyze cephamycins and cephalosporins but are not inhibited by CVA (David et al., 2006).

Salmonellae have been reported to express different types of ESBL types such as TEM, SHV, PER, OXA and CTX-M enzymes (AitMhand et al., 2002; Baraniak et al., 2002; Bradford et al., 1998; Casin et al., 2003; Hanson et al., 2002; Revathi et al., 1998; Villa et al., 2000) as well

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as plasmid-mediated AmpC-type beta-lactamases (Hanson et al., 2002; Pitout et al., 2003; Rankin et al., 2002). More than 340 beta-lactamases have been described in *Salmonella* (Hasman et al., 2005) and the prevalence of genes encoding for them varies region by region (Winokur et al., 2001). There is however a paucity of information on the genes encoding these beta-lactamases, despite resistance to beta-lactam drugs in *Salmonella* isolated from humans and food animals in Nigeria (Ogunleye et al., 2005, 2010b). The current study characterizes five multidrug resistant *Salmonella* isolates: four isolated from septic poultry in Nigeria (Ogunleye et al., 2010a) and one isolated from one out of two hundred asymptomatic healthy pigs from the commercial section of the University of Ibadan Teaching and research farm. Isolates were screened for fourteen possible genes encoding a variety of beta-lactamases responsible for resistance to some third generation cephalosporins that are still very much in use for treatments of human infection in Nigeria.

Our results identified some genes encoding beta-lactamases capable of causing transferable resistance in animals and human, thus constituting a potential public health risk.

MATERIALS AND METHODS

The *Salmonella* isolate

Four of the five multidrug resistant *Salmonella* isolates used for this study were isolated from organs of septic poultry in commercial poultry farms in Nigeria (Ogunleye et al., 2010a, b). The other isolate originated from one out of two hundred asymptomatic pigs screened from the commercial section of the University of Ibadan Teaching and Research Farm in Nigeria. Bacteria were isolated according to standard methods (Barrow and Feltham, 1993).

Serotyping of the isolate

The isolates were sub-cultured into TSA agar and submitted to National Veterinary Service Laboratories in Ames, Iowa, USA for serotyping. Serotyping was performed as per the Kauffman-White Scheme.

Determination of resistance to cefotaxime, ceftazidime, cefovecin, ceftriaxone, cefpirome and ceftiofur

Bacteria were grown aerobically in breakpoint concentrations (32 µg/ml) of cefotaxime, ceftazidime, cefovecin, ceftriaxone, cefpirome and ceftiofur (all obtained from SIGMA-ALDRICH) according to standard method (CLSI, 2009) . Resistance was ascribed if floccu-lent growth was observed after 16 h of aerobic growth at 37°C.

Isolation of cosmid plasmid DNA

Plasmid DNA was isolated from each of the five resistant *Salmonella* isolates using QIAGEN(R) Large-Construct Kit (QIAGEN Companies) as per the manufacturer's protocol.

Amplification of the genes encoding 3rd generation cephalosporin resistance

Fourteen sets of primers (forward and reverse oligosequences shown in Table 1), targeted the following gene classes: TEM, SHV, CTX, PER, DHAM, VEB, GES, OXA2, ACCM, CITM, FOXM, ECBM, MOX and OXA10 were used to amplify the respective genes from plasmid DNA. PCR was performed in a 50 l reactions containing 5 l of 10X Buffer, 3 l of 1.5 mM $MgCl_2$, 3 l each of 250 µM of each deoxynucleoside triphosphate, 2 l each of 10 pmol of the respective forward and reverse primers, 0.5 l of Taq polymerase and 2 l of Cosmid plasmid DNA template and 32.5 l of water. BIO-RAD MJ mini personal thermal cycler was used for the DNA amplification using the following PCR protocol: initial denaturation at 95°C for 2 min, followed by 40 cycles of 95°C for 1 min, 53°C for 30 s and 70°C for 45 s. Amplified DNA products were resolved using 1% (w/v) agarose gel electrophoresis.

RESULTS

Serotype analysis showed two of the four poultry isolate as *S. enterica* serotype Kentucky. One of the two was on-ly resistant to cefpirome at 32 µg/ml breakpoint, while in addition the second one was also resistant to ceftazidime as shown in Table 2. The remaining two poultry isolates were 9, 12: Nonmotile presumably, *Salmonella enterica* serotype Pullorum that have lost their respective 1 antigens due to sub-culturing. One of them was resistant to ceftriaxone, ceftiofur and cefpirome, whereas the second one was only resistant to cefpirome (Table 2). The *Salmonella* isolate from one of the apparently healthy pigs was *S. enterica* serotype Give and was only resistant to ceftriaxone at 32 µg/ml breakpoint. As shown in Figure 1, PCR studies revealed that of the fourteen gene screened for in the five *Salmonella* isolates, *S. enterica* serotype Give from pig contained: TEM, SHV, GES, OXA2, ACCM, FOX and ECBM. The cefpirome resistant *S. enterica* serotype Kentucky from poultry possessed: TEM, SHV, DHAM, OXA2, ECBM, whereas the *Salmonella* Kentucky resistant to both ceftazidime and cefpirome does not carry any of the fourteen genes screened. Also the cefpirome resistant *Salmonella* Pullorum strain possessed: TEM, SHV, DHAM, GES and ECBM, while ceftriaxone, ceftiofur and cefpirome resistant strain does not possess any of the fourteen genes.

DISCUSSION

Salmonella organisms have been reported to express varieties of extended spectrum beta-lactamases

Some beta-lactamases that have been described in *Salmonella* include TEM, SHV, CTX-M, PER and OXA families, as well as CMY-2 AmpC-type (Armand-Lefevre et al., 2003; Hanson et al., 2002). Sometimes some of these genes can occur in multiples in a single isolate

Table 1. Fourteen beta- lactamases gene targeted in the study, the primers oligosequences and related beta-lactamases.

Resistance gene	Base pairs number	Oligosequence	Related enzyme(s)
TEM	931	F = 5´TCCGGTCATGAGACAATAACC3´ R = 5´TTGGTCTGACAGTTACCAATGG3´	TEM1-TEM190.
SHV	868	F = 5´TGGTTATGCGTTATATTCGCC3´ R = 5´GGTTAGCGTTGCCAGTGCT3´	SHV1-SHV63
CTX	909	F = 5´TCTTCCAGAATAAGGAATCCC3´ R = 5´CCGTTTCCGCTATTACAAA3´	CTX-M-1-CTX-M-82.
PER	927	F = 5´ATGAATGTCATCACAAAATG3´ R = 5´TCAATCCGGACTCACT3´	PER1 and PER2
DHAM	405	F = 5´AACTTTCACAGGTGTGCTGGGT3´ F = 5´CCGTACGCATACTGGCTTTGC3´	DHA1 and DHA2.
VEB	914	F = 5´GATAGGAGTACAGACATATG3´ R = 5´TTTATTCAAATAGTAATTCCACG3´	VEB1 and VEB2.
GES	864	F = 5´ATGCGCTTCATTCACGCAC3´ R = 5´CTATTTGTCCGTGCTCAGG3´	GES1and GES 2.
OXA-2	478	F = 5´AAGAAACGCTACTCGCCTGC3´ R = 5´CCACTCAACCCATCCTACCC3´	OXA1, OXA2 andOXA15.
ACCM	346	F = 5´AACAGCCTCAGCAGCCGGTTA3´ R = 5´TTCGCCGCAATCATCCCTAGC3´	ACC only.
CITM	462	F = 5´TGGCCAGAACTGACAGGCAAA3´ R = 5´TTTCTCCTGAACGTGGCTGGC3´	LAT1-LAT4, CMY2-CMY7, BIL1.
FOXM	190	F = 5´AACATGGGGTATCAGGGAGATG3´ R = ´CAAAGCGCGCGTAACCGGATTGG3´	FOX1-FOX5b.
ECBM	302	F = 5´TCGGTAAAGCCGATGTTGCGG3´ R = 5´CTTCCACTGCGGCTGCCAGTT3´	MIR-1T and ACT-1.
MOX M	520	F = 5´GCTGCTCAAGGAGCACAGGAT3´ R = 5´CACATTGACATAGGTGTGGTGC3´	MOX1, MOX2, CMY-1, CMY8-CMY11.
OXA 10	720	F = 5´GTCTTTCGAGTACGGCATTA3´ R = 5´ATTTTCTTAGCGCAACTTAC3´	OXA10, OXA17, OXA56, OXA79, OXA16, OXA14.

(Armand-Lefevre et al., 2003; Hanson et al., 2002). All across the globe, there has been various reports incriminating *Salmonella* serotypes like *S. enterica* serotype Typhimurium, *S. enterica* serotype Seftenberg, *S. enterica* subspecies *enterica* serotype othmarschem pro-ducing TEM, SHV, PER and CTX related beta lactamases in nosocomial infections. For instance, in Tunisia, Turkey, Morocco, Algeria, South Korea, and United Kingdom, such reports have been documented (AitMhand et al., 2002; Bouallegue-Godet et al., 2005; Hammami et al., 1991; Morosini, 1996; Naas et al., 2005; Yong et al., 2005). This study describes the characterization of five *S.*

Table 2. *Salmonella* serotypes studied their animal sources and the respective 3rd generation cephalosporin antibiotic resistance pattern.

Salmonella serotype	Animal source	Antibiogram
Give	pig	Ceftriaxone
Kentucky	Poultry	Cefpirome
Pullorum (presumptive)	Poultry	Cefpirome
Pulorum (presumptive)	Poultry	Ceftriaxone, Cefpirome, Ceftiofur
Kentucky	Poultry	Ceftazidime, cefpirome

The *Salmonella* Pullorum in this study were serotyped as 9, 12: NM, which we presume to be pullorum in which the 1 antigen was lost during subculturing.

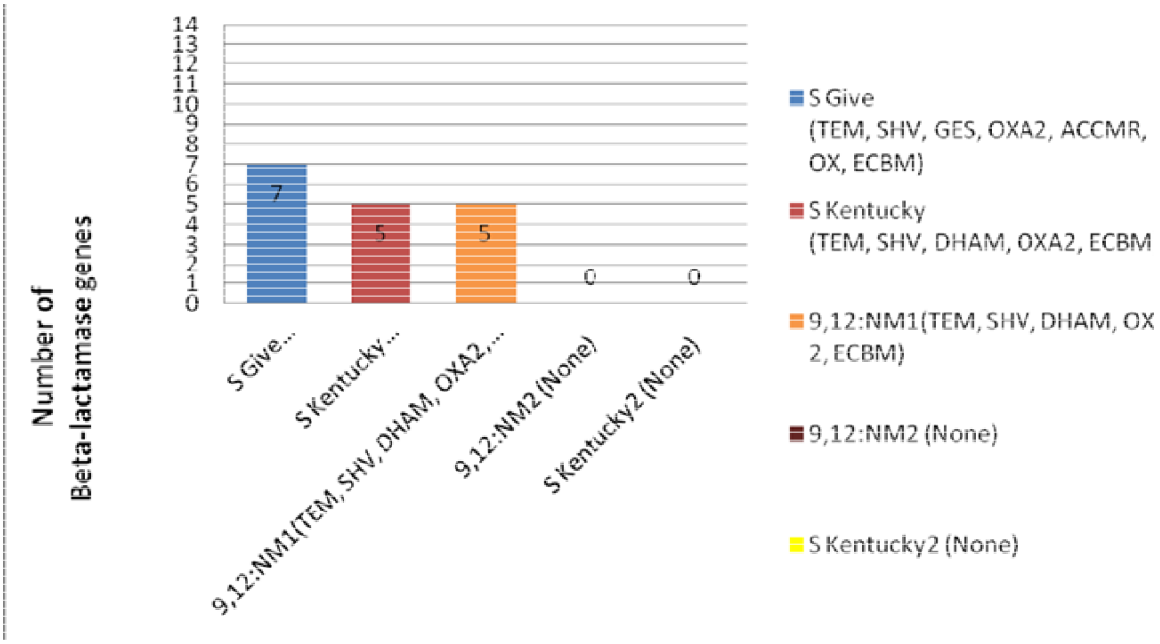

Figure 1. Illustration of the respective genes amplified by the primers in the five *Salmonella* serotypes isolated from poultry and pig from Nigeria.

enterica serotypes isolated from septic poultry and from one out of two hundred apparently healthy pigs in Nigeria (Ogunleye et al., 2010a, b). The characterization was based on some genes (n = 14) responsible for 3rd generation cephalosporin resistance as shown in Table 1. The ceftriaxone resistance observed in the *Salmonella* Give from this study (Table 2) could have been conferred by any of the genes present in this isolate, namely: TEM, SHV, GES, OXA2, ACCM, FOX, ECBM as shown in Figure 1 or due to a novel TEM. All these genes having been earlier reported in some cephalosporin resistant *Salmonella* serotypes such as: *Salmonella* serotypes Wien (Hammami et al., 1991), Typhimurium (Garbarg-Chenon et al., 1989), Mbandaka (Issak et al., 1995), *Salmonella* serotype Typhimurium (AitMhand et al., 2002), *Salmonella* serotype Senftenberg (Koeck et al., 1997), and in *Salmonella* serotype Livingstone (Rhimi-Mahjoubi et al., 2002) in North Africa for example.

The cefpirome resistances in one of the *Salmonella* Kentucky and one *Salmonella* Pullorum are thought to be due to the presence of TEM genes present in these isolates, while other genes like SHV, DHAM, GES, ECBM and OXA-2 are inactive for the cefpirome resistance. However, the non observance of any of the fourteen screened genes in the ceftazidime and cefpirome resistant *Salmonella* Kentucky, as well as in ceftriaxone and ceftiofur resistant *Salmonella* Pullorum as indicated in Table 2 and Figure 1 is suggestive of the fact that the resistance in these two isolates may have been conferred by some other unique gene(s). Since the emergence of *Salmonella* isolates harbouring extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases, it has grown to be a major public health problem worldwide (Bonnet, 2004). Resistance to third-generation

Beta-lactams in *Salmonella* which often results from the production of plasmid-mediated Ambler class A or C (AmpC type) extended- spectrum beta-lactamases (ESBLs) has been reported worldwide (Parry, 2003). In Nigeria however, there are paucity of such reports both in *Salmonella* serotypes from human and food animal origin. This work thus provides an initial database for genes responsible for 3rd generation cephalosporin resis-tance in *Salmonella* strains isolated from food animals from some parts of South Western states of Nigeria. The findings in this work expose the possible health risk in terms of transfer of drug resistance from these food animal to man. Third-generation cephalosporins are still the drug of choice in treating some life threatening infections in Nigeria.

Should these resistance be transferred from food animals to man, it can jeopardize success of effective treatment thus constituting a potential grave public health hazard.

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REFERENCES

AitMhand R, Soukri A, Moustaoui N, Amarouch H, ElMdaghri N, Sirot D, Benbachir M (2002). Plasmid mediated TEM-3 extended-spectrum beta-lactamase production in *Salmonella typhimurium* in Casablanca. J. Antimicrob. Chemother., 49:169-172.

Armand-Lefevre L, Leflon-Guibout V, Bredin J, Barguellil F, Amor A, Page`s JM, Nicolas-Chanoi MH (2003). Imipenem resistance in *Salmonella enterica* serovar Wien related to porin loss and CMY-4 beta-lactamases production. Antimicrob. Agents Chemother., 47: 1165-1168.

Baraniak A, Sadowy E, Hryniewicz W, Gniadkowski M (2002). Two different extended-spectrum beta-lactamases (ESBLs) in one of the first ESBL producing *Salmonella* isolates in Poland. J. Clin. Microbiol., 40: 1095-1097.

Barrow GH, Feltham RKA (1993). Cowan and Steel's manual for identification of medical bacteria. Third edition. Cambridge University Press, Cambridge, 331 p.

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

Bouallegue-Godet O, Ben Salem Y, Fabre L, Demartin M, Grimont PA, Mzoughi R, Weill FX (2005). Nosocomial outbreak caused by *Salmonella enterica* serotype Livingstone producing CTX-M-27 extended-spectrum beta-lactamase in a neonatal unit in Sousse,Tunisia. J. Clin. Microbiol., 43: 1037-1044.

Bradford PA (2001). Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev., 14: 933-951.

Bradford PA, Yang Y, Sahm D, Grope I, Gardovska D, Storch G (1998). CTX-M-5, novel cefotaxime-hydrolyzing beta-lactamase from an outbreak of *Salmonella typhimurium* in Latvia. Antimicrob. Agents Chemother., 42: 1980-1984.

Casin I, Hanau-Berc¸ot B, Podglajen I, Vahaboglu H, Collatz E (2003). *Salmonella enterica* serovar Typhimurium *bla*PER-1-carrying plasmid pSTI1 encodes an extended-spectrum aminoglycoside 6-*N*-acetyltransferase of type Ib. Antimicrob. Agents Chemother., 47: 697-703.

Clinical and Laboratory Standards Institute (2009) M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. 8th ed. Wayne, PA.

Garbarg-Chenon A, Vu Thien H, Labia R, Ben Yaglane H., Godart V Deny P, Bricout F, Nicolas JC (1989).Characterization of a plasmid coding for resistance to broad-spectrum cephalosporin's in *Salmonella typhimurium*. Drugs Exp. Clin. Res., 15: 145-150

Hammami A, Arlet G, Ben Redjeb S, Grimont F, Ben Hassen A, Rekik A, Philippon A (1991). Nosocomial outbreak of acute gastroenteritis in a neonatal intensive care Unit in Tunisia caused by multiply drug resistant *Salmonella enterica* serotype Wien producing SHV-2 beta-lactamase. Eur. J. Clin. Microbiol. Infect. Dis., 10: 641-646.

Hanson ND, Moland ES, Hossain A, Neville SA, Gosbell IB, Thomson KS (2002). Unusual *Salmonella enterica* serotype Typhimurium isolate producing CMY-7, SHV-9 and OXA-30 beta-lactamases. J. Antimicrob. Chemother., 49: 1011-1014.

Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM (2005). Beta-Lactamases among extended-spectrum beta-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. J. Antimicrob. Chemother., 56: 115–121.

Hohmann EL (2001). Nontyphoidal salmonellosis. Clin. Infect. Dis., 32: 263-269.

Issak MI, Shannon KP, Qureshi S A, French GL **(**1995). Extended-spectrum beta -lactamases in *Salmonella*. J. Hosp. Infect., 30: 319–321.

Koeck JL, Arlet G, Philippon A, Basmaciogullari S, Vu Thien H, Buisson Y, Cavallo JD (1997). A plasmid-mediated CMY-2 beta–lactamase from an Algerian clinical isolate of *Salmonella senftenberg*. FEMS Microbiol. Lett., 152: 255-260.

Morosini MI, Blazquez J, Negri MC, Canton R, Loza E, Baquero F (1996). Characterization of a nosocomial outbreak involving an epidemic plasmid encoding for TEM- 27 in *Salmonella enterica* subspecies enterica serotype Othmarschen. J. Infect. Dis., 174: 1015-1020.

Naas T, Lezzar A, Bentchouala C, Smati F, Scheftel JM, Monteil H, Nordmann P(2005). Multidrug-resistant *Salmonella enterica* serotype Senftenberg isolates producing CTX-M Beta-lactamases from Constantine, Algeria. J. Antimicrob Chemother., 56: 439-440.

Ogunleye AO, Ajuwape ATP, Adetosoye AI (2010a). Fluoroquinolone resistant *Salmonella enterica* of poultry origin from South Western States of Nigeria. Bull. Ani. Health Prod. Afr., 58: 216-221.

Ogunleye AO**,** Ajuwape ATP, Adetosoye AI (2010b). Infectious drug resistance plasmid study in *Salmonella enterica* isolates of domestic animals and lizards from some South-Western States of Nigeria. Afr. J. Biotechnol., 9(25): 3726-3731.

Ogunleye VO, Ogunleye AO, Ajuwape ATP, Olawole OM, Adetosoye AI (2005). Childhood septicaemia due to *Salmonella* species in Ibadan, Nigeria. AJBR., 8: 131-134.

Parry C (2003). Antimicrobial drug resistance in *Salmonella enterica*. Curr. Opin. Infect. Dis., 16: 467-472.

Pitout JD, Reisbig MD, Mulvey M, Chui L, Louie M, Crowe L, Church DL, Elsayed S, Gregson D, Ahmed R, Tilley P, Hanson ND (2003). Association between handling of pet treats and infection with *Salmonella* enterica serotype Newport expressing the AmpC beta-lactamases, CMY-2. J. Clin. Microbiol., 41: 4578-4582.

Rankin SC, Aceto H, Cassidy J, Holt J, Young S, Love B, Tewari D, Munro DS, Benson CE(2002). Molecular characterization of cephalosporin-resistant *Salmonella enterica* serotype Newport isolates from animals in Pennsylvania. J. Clin. Microbiol., 40: 4679-4684.

Revathi G, Shannon KP, Stapleton PD, Jain BK, French GL (1998). An outbreak of extended-spectrum, beta-lactamase-producing *Salmonella senftenberg* in a burns ward. J. Hosp. Infect., 40: 295-302.

Rhimi-Mahjoubi F, Bernier M, Arlet G, Ben Jemaa Z, Jouve P,

Hammami A, Philippon A (2002). Mise en e´vidence de la ce´phalosporinase plasmidique ACC-1 dans diffe´rentes ente´robacte´ries (*Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella*) isole´es dans un Hoˆpital Tunisien (Sfax 1997– 2000). Pathol. Biol., 50: 7-11

Shahada F, Chuma T, Dahshan H, Akiba M, Sueyoshi M, Okamoto K (2010). Detection and Characterization of Extended-Spectrum beta-Lactamase (TEM-52 Producing *Salmonella enterica* Serotype Infantis from Broilers in Japan. Food borne Path. Dis., 7(5): 515-521.

Threlfall EJ (2002). Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. FEMS Microbiol Rev., 26: 141-148.

Villa L, Pezzella C, Tosini F, Visca P, Petrucca A, Carattoli A (2000). Multiple-antibiotic resistance mediated by structurally related IncL/M plasmids carrying an extended-spectrum beta-lactamase gene and a class 1 integron. Antimicrob. Agents Chemother., 44: 2911-2914.

Winokur PL, Canton R, Casellas JM, Legakis N (2001). Variations in the prevalence of strains expressing an extended-spectrum beta-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific region. Clin. Infect. Dis., 32(Suppl. 2): S94-103.

Yong A, Lim YS, Yum JH, Lee H, Lee K, Kim EC, Lee BK, Chong Y (2005). Nosocomial outbreak of pediatric gastroenteritis caused by CTX-M-14-type extended-spectrum beta-lactamase-producing strains of *Salmonella enterica* serovar London. J. Clin. Microbiol., 43: 3519-3521.