Characterization of Extended-Spectrum Beta-Lactamase–Producing Salmonella enterica Serotype Brunei and Heidelberg at the Hussein Dey Hospital in Algiers (Algeria)

Rachida Kermas, Abdelaziz Touati, Lucien Brasme, Elisabeth Le Magrex-Debar, Sadjia Mehrane, François-Xavier Weill, Christophe de Champs

Nosocomial outbreaks of extended-spectrum beta-lactamase-producing *Salmonella enterica* serotype Brunei and Heidelberg at the Hussein Dey hospital in Algiers (Algeria)

Rachida KERMAS[1], **Abdelaziz TOUATI[1],** Lucien BRASME[3], Elisabeth LE MAGREX-DEBAR[3], Sadjia MEHRANE[2], François-Xavier WEILL[4], and Christophe DE CHAMPS[3]

[1] Département de microbiologie, FSNV, Université A/MIRA de Bejaia 06000, Algeria

[2] Laboratoire central de biologie clinique, CHU HUSSEIN DEY, Alger, Algeria

[3] Laboratoire de Bactériologie–Virologie-Hygiène Hospitalière, CHU Reims, Hôpital Robert DEBRE, Avenue du Général Koenig, 51092 Reims Cedex, France

[4] Institut Pasteur, Centre national de référence des Salmonella, Unité des Bactéries Pathogènes Entériques, 28 rue du docteur Roux, Paris cedex 15, France.

Corresponding author's

Abdelaziz TOUATI

Département de microbiologie, FSNV, Université A/MIRA de Béjaia, 06000, Algeria

Mail: ziz1999@yahoo.fr

Tel/fax : 213.34214762

Abstract

Objectives: The purpose of this work was to study the genetic determinants responsible for extended-spectrum β-lactamase (ESBL) resistance of *Salmonella* isolated from children during the period1995 to 2008 at the Hussein Dey hospital in Algiers (Algeria).

Methods: Fourteen ESBL-resistant *Salmonella* isolates were tested towards 22 antimicrobial agents. PCR and sequencing were used to determine the underlying genetic determinants responsible for the ESBL phenotypes. The transferability of the ESBL phenotypes was tested by conjugation, and ERIC-PCR was employed to typing the isolates.

Results: All isolates were resistant to ticarcillin, ticarcillin-clavulanate, piperacillin, amoxicillin-clavulanate, cefuroxime, aztreonam, ceftazidime, cefotaxime (except 2 isolates), cefepime and cefpirome. PCR and DNA sequencing identified these extended-spectrum β-lactamases as TEM-48 (n=6), TEM-4 (n=3), CTX-M-15 (n=4), and one new TEM, designated TEM-188.

Conclusion: this study demonstrates the emergence of a public health risk related to ESBL in *Salmonella* in Algeria.

Keywords:

Outbreaks, Children, *Salmonella* Brunei, Heidelberg, Extended-spectrum β-lactamase, TEM-188

INTRODUCTION

Non-typhoidal salmonellae are one of the principal pathogens implicated in food-borne gastroenteritis worldwide. Animals and their products, particularly meat, chicken eggs, and milk, are major sources of human infection. The incidence of non-typhoidal *Salmonella* infections has increased considerably in many countries, but with marked differences among countries (Makanera et *al.,* 2003). Although antibiotics are not usually recommended in cases of *Salmonella* Enterocolitis, they are crucial in systemic infections. Extra-intestinal infectious complications, including meningitis, sepsis and bacteraemia are more common in infants and the elderly, and in immunocompromised patients. In these potentially life-threatening cases, the antibiotics of choice are fluoroquinolones and extended-spectrum cephalosporins. *Salmonella spp*. resistant to extended-spectrum cephalosporins have been recognized since 1988, and are increasing in prevalence worldwide. This is of particular concern for the treatment of salmonellosis in children, because fluoroquinolones should not be used in this age group (Kruger et al., 2004 ; Yates and Amyes, 2005).

To date, more than 340 β-lactamases have been described. *Salmonella* have been found to express a wide variety of ESBL types, including TEM (Ait Mhand *et al.,* 2002), SHV (Hammami *et al.,* 1991), PER (Casin *et al*., 2003), OXA (Hanson *et al.,* 2002), and CTX-M (Tamang *et al.*, 2011), and, more recently, plasmid-mediated AmpC type enzymes, such as DHA-1 (Barnaud *et al*., 1998), CMY-2 (Koeck *et al.,* 1997), and ACC-1 (Rhimi-Mahjoubi *et al.,* 2002).

In Algeria, ESBLs have been identified in nosocomial isolates of various *Enterobacteriaceae*, such as *E. coli*, *K. pneumoniae* and *E. cloacae* (Touati *et al*., 2006; Iabadene *et al*., 2008; Messai *et al*., 2008, Ramdani-Bouguessa *et al*., 2011). However, only few reports for the presence of these enzymes in *Salmonella* have been published (Naas *et al*., 2005; Touati *et al*., 2008; Iabadene *et al*., 2009; Naas *et al*., 2011; Bouzidi *et al.*, 2011).

In this study, we characterized the ESBLs in a collection of putative ESBL positive *Salmonella* spp. isolated from 1995 to 2008 at the Hussein Dey hospital in Algiers (Algeria).

METHODS

Bacterial isolates

A collection of 14 non-duplicate amoxicillin-resistant *S. enterica* isolates was examined. They were obtained from stool samples of children between 1995 and 2008 at the Hussein Dey hospital in Algiers (Algeria). All isolates were biochemically identified by using the API 20E identification system (BioMérieux, Marcy l'Étoile, France).

All isolates were serotyped at the French National Reference Center for Salmonella, Institut Pasteur, Paris, France on the basis of somatic O, phase 1 flagellar, and phase 2 flagellar antigens by agglutination tests with antisera (BioRad, Marnes-la-coquette, France). The serotypes were designated according to the White-Kauffman-Le Minor scheme.

Susceptibility testing and ESBL detection

Disk diffusion susceptibility tests for aztreonam, ticarcillin, piperacillin, amoxicillin-clavulanate, ticarcillin-clavulanate, cefoxitin, cefpirome, cefepime, piperacillin-tazobactam, cefuroxime, imipenem, tobramycin, amikacin, gentamicin, kanamycin, sulfonamide, trimethoprim-sulfamethoxazole, nalidixic acid, ciprofloxacin, tetracycline and chloramphenicol (BioRad) were performed according to the recommendations of the Antibiogram Committee of the French Society for Microbiology (http://www.sfm.asso.fr/). Minimum inhibitory concentrations (MICs) for amoxicillin, cefotaxime, ceftazidime and ceftriaxone were determined by Etest (AB BIODISK, Solna, Sweden) performed on Mueller–Hinton agar plates as recommended by the manufacturer.

Extended-Spectrum β-Lactamase (ESBL) production was detected by a double-disk synergy test (DDST) and was performed by placing disks of ceftazidime, cefotaxime

and aztreonam at a distance of 20mm (centre to centre) from a disk with amoxicillin/clavulanic acid (20/10 µg). Enhancement of the inhibition zone between the disks containing clavulanic acid and cefotaxime, ceftazidime or aztreonam indicated the ESBL production (Jarlier *et al.*, 1988).

β-Lactamases characterization

Total DNA was extracted by using the QIAmp DNA mini kit (Qiagen, Courtaboeuf, France) according to the instructions of the manufacturer. The ESBL-encoding genes bla_{TEM}, bla_{SHV} and bla_{CTX-M} were detected by PCR using specific primers (Table 1) and further identified by nucleotide sequence analysis of the PCR products. Sequences were analyzed using the BLAST 2.0 (Basic Local Alignment Search Tool) software available on the website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast/BLAST.cgi)

PCR fingerprinting

For enterobacterial repetitive intergenic consensus (ERIC) PCR, whole-cell DNA of isolates was extracted using the QIAmp DNA mini kit (Qiagen). The primers were ERIC1 (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTACTGGGGTGAGCG-3'). Each 23 µl PCR reaction mixture contained 25 pmol of each primer, 5mmol/L each deoxynucleotide triphosphate (dNTP) and 2.5 U of Taq polymerase (Qiagen, Courtaboeuf, France) in the manufacturer's provided buffer. Two microliters of total DNA (about 80 ng) was added to each reaction to reach the final 25µl PCR reaction volume. The ERIC-PCR parameters were as follows: initial denaturation at 95°C for 7 min; 30 cycles of denaturation at 92°C for 30 s, annealing at 50°C for 1 min, and extension at 65°C for 8 min; followed by a final extension at 65°C for 16 min (Cao *et al.*, 2008**)**. PCR amplicons were resolved

on 1% agarose gel containing ethidium bromide by horizontal electrophoresis in Tris-borate-EDTA buffer. Gels were visualized under UV light with Bio-Profil (Vilbert Lourmat, Torcy, France).

RESULTS

Fourteen strains belonging to *Salmonella enterica* serotypes Brunei (10 strains) and Heidelberg (4 strains) were isolated and serotyped in the laboratory and confirmed at the Pasteur Institute in Paris, France. Te strains were isolated from infants.

All isolates exhibited resistance or decreased susceptibilities to ticarcillin, ticarcillin-clavulanate, piperacillin, amoxicillin-clavulanate, cefuroxime, aztreonam, ceftazidime, cefotaxime, cefepime and cefpirome (table 2). They remained susceptible to imipenem, cefoxitin and piperacillin-tazobactam. The DDS test was positive for all of these isolates. MICs determination showed that S. Brunei examined (Table 3) were resistant to amoxicillin (MIC > 256µg/ml), ceftazidime (MIC = 64µg/ml), cefotaxime (MIC = 16µg/ml) and ceftriaxone (MIC = 6µg/ml). For S. Heidelberg strains, resistance were observed for all strains (amoxicillin: MIC > 256µg/ml, ceftazidime: MIC = 48µg/ml, cefotaxime: MIC > 32µg/ml and ceftriaxone: MIC > 32µg/ml)

All isolates were resistant to gentamicin and tobramycin. The isolates of *S.* Brunei were resistant to kanamycin and amikacin (except isolates of S9 and S12). Resistance to nalidixic acid was observed in all isolates of *S.* Heidelberg. All of the strains were susceptible to ciprofloxacin, cotrimoxazole, tetracycline and chloramphenicol.

TEM consensus PCR assays gave the expected PCR fragments for the 10 strains of *S.* Brunei (Table 2) and CTX-M amplifications were positive for the 4 strains of *S.* Heidelberg. SHV amplification was negative.

Three isolates of *S.* Brunei (S9, S12 and S22) harbored the bla_{TEM-4} gene and the bla_{TEM-48} gene was found in six isolates of S. Brunei (S15, S16, S18, S20, S21 and S23). The four strains of *S.* Heidelberg were found to produce CTX-M-15.

One strain of *S.* Brunei (S10) was found to produce a new TEM. This protein has been designated TEM-188 (http:www.lahey.org/studies/webt.htm, GenBank Accession Number JN211012). The new TEM β-lactamase differed from TEM-1 by three substitutions: Leu21Phe, Gly238Ser and Glu240Lys. These substitutions are identical to those found in TEM-48. However, TEM-48 has an additional substitution, Thr265Met. Isolates producing TEM-48 and TEM-188 showed identical antibiotypes (table 2 and 3) suggesting that the substitutionThr265Met in TEM-48 has no effect on β-lactams susceptibility.

The ERIC-PCR method was applied to the six TEM-48-producing *S.* Brunei strains and the four CTX-M-15-producing-*S*.Heidelberg. Two ERIC-PCR patterns were observed, one for the six *S.* Brunei and a second one for the four *S*. Heidelberg, suggesting a clonal expansion for each resistant population defined by its serotype.

Discussion

There are a number of commonly identified serotypes of *Salmonella* associated with human infections. In the United States, the most common serovars were Typhimurium, Enteritidis, Newport, Heidelberg, and Javiana. In other parts of the world, there are some differences in the predominant serovars associated with disease. In the European Union, Enteritidis is the predominant serovar. In many parts of Asia, Choleraesuis is one of the top serovars (Foley and Lynne, 2007). *S.* Brunei has been rarely reported from animals, animal food products, and patients with human salmonellosis. There are only 3 articles found on pubmed when we use Salmonella Brunei as keyword.

In our study, 10 strains of *S*. Brunei were recovered from infants during the period 1995 to 2008 whereas the 4 *S*. Heidelberg were recovered only in October 2008. Unfortunately, data of the commonly identified serotypes in Algeria were not available.

The largest subset of the population for which antibiotic susceptibility of Salmonella is a major concern is children. Although, gastroenteritis is the most frequent clinical manifestation, systemic infections are common, and even cases of meningitis have been reported. Such serious infections are most common in children and the elderly. Antibiotic therapy is strongly recommended in such cases (Arlet et *al.,* 2006). Extended-spectrum cephalosporins are commonly used to treat patient with invasive infections or severe diarrhea caused by *Salmonellae*; however, during the past years extended-spectrum cephalosporins-resistant *Salmonellae* have frequently been reported worldwide, including north Africa (Ahmed *et al.,* 2009; Ohmani *et al.,* 2010; Bouzenoune *et al.,* 2011; Naas *et al.,* 2011).

The 1st salmonella strains with ESBLs in Africa were identified in 1988 in Tunisia (Hammami et *al.,* 1991). TEM-4 β-lactamase, which differed from the TEM-1 β-lactamase sequence by 4 substitutions (Leu21Phe, Glu104Lys, Gly238Ser and Thr265Met), was first reported for *E*. *coli* in France by Paul et *al.* (Paul *et al.,* 1989) This enzyme was described in an isolate of *Salmonella* collected during a French national survey in 1998 (De Champs *et al.*, 2000) and reported in isolates of *Salmonella* serotype Mbandaka in Tunisia (Makanera *et al.,* 2003). This was the first reported identification of the TEM-4 ESBL in Algerian *Salmonella* Brunei.

The amino acid substitutions of the sequence of TEM-48 compared to the TEM-1 β-lactamase sequence were Leu21Phe, Gly238Ser, Glu240Lys and Thr265Met. TEM-48 was first described in *K. pneumoniae* strains in Poland in Poland (Gniadkowski *et al.*, 1998). To our knowledge, no report on TEM-48 isolated from *Salmonella* has been previously published. Moreover, all isolates of *S*. Brunei producing TEM-48 were resistant to aminoglycosides and sulfonamide.

CTX-M-15 was identified in different salmonella serotypes, but to our knowledge, this is the first report of CTX-M-15 in *S.* Heidelberg isolates. CTX-M-15-producing *Salmonella* isolates were reported in different serotypes in Algeria, including Infantis (Naas et *al.,* 2011) and Kedougou (Touati et *al.,* 2008). The four isolates of *S*. Heidelberg were found resistant to nalidixic acid, but susceptible to fluoroquinolones. PCR for the plasmid-mediated mechanisms were negative for the four isolates, suggesting that the nalidixic resistance was probably mediated by mutations in topoisomerases.

PCR-mediated genome fingerprinting based on ERIC or REP has been found useful for the typing of outbreak and sporadic *Salmonella* isolates (Merino *et al.*, 2003).

Nosocomial outbreaks due to ESBLs-producing *Salmonella* have been described in many countries, such as the outbreak in Tunisia due to TEM-4-producing S. Mbandaka (Makanera et *al.,* 2003). The great majority of them have involved pediatric wards and especially neonatology units. In the community, many outbreaks have been reported and were largely foodborne outbreaks (Arlet et *al.,* 2006). The two clonal strains observed in our study, were recovered throughout the 13-year study period. The *S.* Brunei-producing TEM-48 strains were recovered from neonatology ward, except one strain recovered from cradle ward, whereas the S. Heidelberg-producing CTX-M-15 strains were isolated in cradle ward in which the age of children is about 3 months. These observations indicated that gastrointestinal infections were caused mainly by clonally related *Salmonella* serotype isolates and clonal spread was responsible for their dissemination.

Salmonellosis is most often attributed to the consumption of contaminated foods such as poultry, beef, pork, eggs, milk, seafood, nut products, and fresh produce (Foley and Lynne, 2007). In this outbreak, food as a source was excluded because milk was commercially prepared and other infants, hospitalized in the same ward at the same period, were fed with the same preparations but did not become infected with these strains. Therefore, a horizontal transmission of the outbreak strain had probably occurred.

In conclusion, this study demonstrates the emergence of a public health risk related to β-lactams resistance in *Salmonella* in Algeria. The implementation of effective screening methods for the detection of beta-lactamases and ESBLs as well as the establishment of surveillance programs became key factors in the control of hospital outbreaks.

Acknowledgments

We thank Janick Madoux for her technical assistance.[3]

References

Ahmed, A.M, Younis, E.E., Ishida, Y. & Shimamoto, T. (2009). Genetic basis of multidrug resistance in *Salmonella enterica* serovars Enteritidis and Typhimurium isolated from diarrheic calves in Egypt. Acta Trop **111**:144-9

Ait Mhand, R., Soukri, A., Moustaoui, N., Amarouch, H., El Mdaghri, 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.

Arlet G, Barrett TJ, Butaye P, Cloeckaert A, Mulvey MR, White DG. **Salmonella** resistant to extended-spectrum cephalosporins: prevalence and epidemiology. Microbes Infect. 2006 Jun;8(7):1945-54

Barnaud, G., Arlet, G., Verdet, C., Gaillot, O., Lagrange, P.H. & Philippon A. (1998). *Salmonella enteritidis* AmpC plasmid-mediated inducible beta-lactamase (DHA-1) with an ampR gene from *Morganella morganii*. Antimicrob Agents Chemother **42**:2352-8.

Bouzidi, N., Aoun, L., Dekhil, M., Granier, S.A., Poirel, L., Brisabois, A., Nordmann, P. & Millemann, Y. (2011). Co-occurrence of aminoglycoside resistance gene *armA* in non-Typhi Salmonella isolates producing CTX-M-15 in Algeria. J Antimicrob Chemother **66**:2180-1.

Bouzenoune, F., Kellab Debbih, K., Boudersa, F., Kouhil, S. & Nezzar, N. (2011). Antibiotic susceptibility of *Salmonella enterica* serovar Typhi isolated from blood cultures at the Ain M'lila hospital (Algeria), between 2005 and 2008. Med Mal Infect **41**:181-5.

Brasme, L., Nordmann, P., Fidel, F., Lartigue, M.F., Bajolet, O., Poirel, L., Forte, D., Vernet-Garnier, V., Madoux, J. & other authors. (2007). Incidence of class A extended-spectrum beta-lactamases in Champagne-Ardenne (France): a 1 year prospective study.J Antimicrob Chemother **60**:956-64.

Cao, S.Y., Wang, M.S., Cheng, A.C., Qi, X.F., Yang, X.Y., Deng, S.X., Yin, N.C., Zhang, Z.H., Zhou, D.C. & other authors. (2008). Comparative analysis of intestinal microbial community diversity between healthy and orally infected ducklings with *Salmonella enteritidis* by ERIC-PCR. World J Gastroenterol **14**:1120-5.

Casin, I., Hanau-Berçot, 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.

Chanal, C., Bonnet, R., De Champs, C., Sirot, D., Labia, R. & Sirot, J. (2000) Prevalence of beta-lactamases among 1,072 clinical strains of *Proteus mirabilis*: a 2-year survey in a French hospital. Antimicrob Agents Chemother **44**:1930-5.

De Champs, C., Sirot, D., Chanal., C., Bonnet., R. & Sirot J. (2000). A 1998 survey of extended-spectrum beta-lactamases in *Enterobacteriaceae* in France. The French Study Group. Antimicrob Agents Chemother **44:**3177-9.

Dutour, C., Bonnet, R., Marchandin, H., Boyer, M., Chanal, C., Sirot, D. & Sirot J. (2002). CTX-M-1, CTX-M-3, and CTX-M-14 beta-lactamases from *Enterobacteriaceae* isolated in France. Antimicrob Agents Chemother **46**:534-7.

Foley SL, **Lynne** AM, Nayak R. Salmonella challenges: prevalence in swine and poultry and potential pathogenicity of such isolates. J Anim Sci. 2008 Apr;86(14 Suppl):E149-62

Gniadkowski, M., Schneider, I., Jungwirth, R., Hryniewicz, W. & Bauernfeind, A. (1998). Ceftazidime-resistant *Enterobacteriaceae* isolates from three Polish hospitals: identification of three novel TEM- and SHV-5-type extended-spectrum beta-lactamases. Antimicrob Agents Chemother **42:**514-20.

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 wien producing SHV-2 beta-lactamase. Eur J Clin Microbiol Infect Dis **10**:641-6.

Hanson, N.D., Moland, E.S., Hossain, A., Neville, S.A., Gosbell, I.B. & Thomson, K.S. (2002). Unusual *Salmonella enterica* serotype Typhimurium isolate producing CMY-7, SHV-9 and OXA-30 beta-lactamases. J. Antimicrob. Chemother **49**:1011–1014.

Iabadene, H., Messai, Y., Ammari, H., Ramdani-Bouguessa, N., Lounes, S., Bakour, R. & Arlet, G. (2008). Dissemination of ESBL and Qnr determinants in *Enterobacter cloacae* in Algeria. J Antimicrob Chemother **62**:133-6

Iabadene, H., Bakour, R., Messai, Y., Da Costa, A. & Arlet, G. (2009). Detection of blaCTX-M-14 and aac(3)-II genes in *Salmonella enterica* serotype Kedougou in Algeria. Med Mal Infect **39**:806-7

Jarlier, V., Nicolas, M.H., Fournier, G. & Philippon, A. (1988). Extended-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev Infect Dis **10:**867–78.

Koeck, J.L., Arlet, G., Philippon, A., Basmaciogullari, S., Thien, H.V., Buisson., Y. & Cavallo, J.D. (1997). A plasmid-mediated CMY-2 beta-lactamase from an Algerian clinical isolate of Salmonella senftenberg. FEMS Microbiol Lett Jul **15**:255-60.

Kruger, T., Szabo, D., Keddy, K.H., Deeley, K., Marsh, J.W., Hujer, A.M., Bonomo, R.A. & Paterson, DL. (2004). Infections with nontyphoidal Salmonella species producing TEM-63 or a novel TEM enzyme, TEM-131, in South Africa. Antimicrob Agents Chemother **48**:4263-70

Kojima, A., Ishii, Y., Ishihara, K., Esaki, H., Asai, T., Oda, C., Tamura, Y., Takahashi, T. & Yamaguchi, K. (2005). Extended-spectrum-beta-lactamase-producing *Escherichia coli* strains isolated from farm animals from 1999 to 2002: report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. Antimicrob Agents Chemother **49**:3533-7.

Makanera, A., Arlet, G., Gautier, V. & Manai, M. (2003). Molecular epidemiology and characterization of plasmid-encoded beta-lactamases produced by Tunisian clinical isolates of *Salmonella enterica* serotype Mbandaka resistant to broad-spectrum cephalosporins. J Clin Microbiol **7**, 2940-5.

Merino, L.A., Ronconi, M.C., Navia, M.M., Ruiz, J., Sierra, J.M., Cech, N.B., Lodeiro, N.S. & Vila, J. (2003) Analysis of the clonal relationship among clinical isolates of *Salmonella enterica* serotype Infantis by different typing methods. Rev Inst Med Trop Sao Paulo **45 :**119-23.

Messai, Y., Iabadene, H., Benhassine, T., Alouache, S., Tazir, M., Gautier, V., Arlet, G. & Bakour, R. 2008. Prevalence and characterization of extended-spectrum

beta-lactamases in *Klebsiella pneumoniae* in Algiers hospitals (Algeria). Pathol Biol (Paris) **56**:319-25

Naas, T., Lezzar, A., Bentchouala, C., Smati, F., Scheftel, J.M., Monteil, H. & Nordmann, P. (2005). Multidrug-resistant *Salmonella enterica* serotype Senftenberg isolates producing CTX-M beta-lactamases from Constantine, Algeria. J Antimicrob Chemother **56**:439-40

Naas, T., Bentchouala, C., Cuzon, G., Yaou, S., Lezzar, A., Smati, F. & Nordmann, P. (2011). Outbreak of *Salmonella enterica* serotype Infantis producing ArmA 16S RNA methylase and CTX-M-15 extended-spectrum β-lactamase in a neonatology ward in Constantine, Algeria. Int J Antimicrob Agents **38**:135-9

Ohmani, F., Khedid, K., Britel, S., Qasmaoui, A., Charof, R., Filali-Maltouf, A. & El Aouad, R. (2010). Antimicrobial resistance in *Salmonella enterica* serovar Enteritidis in Morocco. J Infect Dev Ctries **4**:804-9.

Paul, G.C., Gerbaud, G., Bure, A., Philippon, A. M., Pangon, B. & Courvalin, P. (1989). TEM-4, a new plasmid-mediated beta-lactamase that hydrolyzes broad-spectrum cephalosporins in a clinical isolate of *Escherichia coli*. Antimicrob. Agents Chemother **33:**1958–1963.

Ramdani-Bouguessa, N., Manageiro, V., Jones-Dias, D., Ferreira, E., Tazir, M. & Caniça, M. (2011). Role of SHV β-lactamase variants in resistance of clinical *Klebsiella pneumoniae* strains to β-lactams in an Algerian hospital. J Med Microbiol **60**:983-7

Rhimi-Mahjoubi, F., Bernier, M., Arlet, G., Jemaa, Z.B, Jouve, P., Hammami, A. & Philippon, A. (**2002).** Identification of plasmid-encoded cephalosporinase ACC-1 among various enterobacteria (*Klebsiella pneumoniae, Proteus mirabilis*, Salmonella) isolated from a Tunisian hospital (Sfax 997-2000). Pathol Biol (Paris) **50**:7-11.

Tamang, M.D., Nam, H.M., Kim, T.S., Jang, G.C., Jung, S.C. & Lim, S.K. (2011). Emergence of extended-spectrum beta-lactamase (CTX-M-15 and CTX-M-14)-producing nontyphoid Salmonella with reduced susceptibility to ciprofloxacin among food animals and humans in Korea. J Clin Microbiol **49**:2671-5

Touati, A., Benallaoua, S., Forte, D., Madoux, J., Brasme, L. & de Champs C. (2006). First report of CTX-M-15 and CTX-M-3 beta-lactamases among clinical isolates of Enterobacteriaceae in Béjaia, Algeria. Int J Antimicrob Agents **27**:397-402.

Touati, A., Benallaoua, S., Gharout, A., Amar, A.A., Le Magrex Debar, E., Brasme, L., Madoux, J., De Champs, C. & Weill, F.X. (2008). First report of CTX-M-15 in Salmonella enterica serotype Kedougou recovered from an Algerian hospital. Pediatr Infect Dis J **27**:479-80.

Yates, C. & Amyes, S. (2005). Extended-spectrum beta-lactamases in non-typhoidal Salmonella spp. isolated in the UK are now a reality: why the late arrival?. J Antimicrob Chemother **56**: 262-4.

364 **Table 1: Primers used in this study**

Target	Primer	Primer sequence	Reference
bla_{CTX-M}	CTX-M1-A2	5′-CTTCCAGAATAAGGAATC-3′	Dutour *et al.*, 2002
	628R	5′-CCTTTCATCCATGTCACCA-3′	Brasme *et al.*, 2007
	405F	5′-GTGGCGATGAATAAGCTGA-3′	Brasme *et al.*, 2007
	CTX-M1-B2	5′-CCGTTTCCGCTATTACAA-3′	Dutour *et al.*, 2002
bla_{TEM}	TEM-A	5′-TAAAATTCTTGAAGACG-3′	Chanal *et al.*, 2000
	TEM-B	5′-TTACCAATGCTTAATCA-3′	
bla_{SHV}	SHV-F	5′-ATGCGTTATATTCGCCTGTG-3′	Kojima *et al.*, 2005
	SHV-R	5′-TTAGCGTTGCCAGTGCTCGA-3′	

Table 2: Characteristics of Algerian clinical isolates of *S.* Brunei and *S.* Heidelberg resistant to broad-spectrum cephalosporins

Isolates	Isolation date	Ward	CXM	TIC	PIP	AMC	FEP	CPO	TCC	ATM	CTX	CAZ	TZP	IMP	FOX	Additional resistance	ESBL type
S. Brunei S10	23/04/1997	Neonatology	19 [R]	6 [R]	6 [R]	19 [I]	22 [I]	21 [I]	19 [R]	12 [R]	25 [I]	12 [R]	25 [S]	35 [S]	30 [S]	GEN, TOB, KAN, AMK	TEM-188
S. Brunei S12	20/09/1994	Neonatology	11 [R]	6 [R]	6 [R]	24 [S]	21 [I]	21 [I]	23 [I]	24 [I]	25 [I]	17 [R]	28 [S]	35 [S]	30 [S]	GEN, TOB, KAN	TEM-4
S. Brunei S15	19/03/2008	Neonatology	16 [R]	6 [R]	6 [R]	19 [I]	22 [I]	20 [I]	19 [R]	12 [R]	23 [I]	10 [R]	23 [S]	35 [S]	31 [S]	GEN, TOB, KAN, AMK, SUL	TEM-48
S. Brunei S16	27/03/2008	Cradle	18 [R]	6 [R]	6 [R]	19 [I]	22 [I]	20 [I]	19 [R]	12 [R]	23 [I]	14 [R]	23 [S]	36 [S]	30 [S]	GEN, TOB, KAN, AMK, SUL	TEM-48
S. Brunei S18	25/06/1998	Neonatology	17 [R]	6 [R]	6 [R]	19 [I]	22 [I]	21 [I]	20 [R]	12 [R]	23 [I]	10 [R]	23 [S]	35 [S]	29 [S]	GEN, TOB, KAN, AMK, SUL	TEM-48
S. Brunei S20	19/03/2008	Neonatology	18 [R]	6 [R]	6 [R]	19 [I]	22 [I]	21 [I]	20 [R]	11 [R]	23 [I]	10 [R]	23 [S]	34 [S]	30 [S]	GEN, TOB, KAN, AMK, SUL	TEM-48
S. Brunei S21	12/12/1995	Neonatology	18 [R]	6 [R]	6 [R]	19 [I]	21 [I]	20 [I]	19 [R]	12 [R]	23 [I]	10 [R]	23 [S]	35 [S]	30 [S]	GEN, TOB, KAN, AMK, SUL	TEM-48
S. Brunei S22	26/07/1998	Neonatology	18 [R]	6 [R]	6 [R]	19 [I]	22 [I]	21 [I]	19 [R]	12 [R]	24 [I]	11 [R]	23 [S]	35 [S]	30 [S]	GEN, TOB, KAN, AMK	TEM-4
S. Brunei S23	12/02/1995	Neonatology	18 [R]	6 [R]	6 [R]	19 [I]	22 [I]	21 [I]	19 [R]	12 [R]	24 [I]	11 [R]	23 [S]	36 [S]	31 [S]	GEN, TOB, KAN, AMK, SUL	TEM-48
S. Brunei S9	22/03/1998	Neonatology	19 [R]	6 [R]	6 [R]	19 [I]	23 [I]	22 [I]	20 [R]	11 [R]	25 [I]	10 [R]	25 [S]	36 [S]	31 [S]	GEN, TOB, KAN	TEM-4
S. Heidelberg S1	26/10/2008	Cradle	6 [R]	6 [R]	6 [R]	24 [S]	11 [R]	9 [R]	22 [R]	12 [R]	11 [R]	14 [R]	26 [S]	36 [S]	31 [S]	GEN, TOB, NAL	CTX-M-15
S. Heidelberg S2	26/10/2008	Cradle	6 [R]	6 [R]	6 [R]	24 [S]	14 [R]	10 [R]	22 [R]	12 [R]	10 [R]	14 [R]	26 [S]	35 [S]	30 [S]	GEN, TOB, NAL	CTX-M-15
S. Heidelberg S3	16/10/2008	Cradle	6 [R]	6 [R]	6 [R]	24 [S]	14 [R]	10 [R]	22 [R]	12 [R]	10 [R]	13 [R]	28 [S]	35 [S]	30 [S]	GEN, TOB, NAL	CTX-M-15
S. Heidelberg S8	03/10/2008	Cradle	6 [R]	6 [R]	6 [R]	24 [S]	13 [R]	10 [R]	25 [R]	12 [R]	8 [R]	14 [R]	26 [S]	35 [S]	30 [S]	GEN, TOB, NAL	CTX-M-15

Legend:

CXM: cefuroxime, **TIC**: ticarcillin, **PIP**: piperacillin, **AMC**: amoxicillin-clavulanate, **FEP**: cefepime, **CPO**: cefprirom, **CTX**: cefotaxim, **CAZ**: ceftazidime, **TZP**: piperacillin-tazobactam, **IMP**: imipenem, **FOX**: cefoxitin, **GEN**: gentamicin, **TOB**: tobramycin, **Kan**: kanamycine, **AMK**: amikacin, **SUL**: sulfonamide, **[R] :** Resistant, **[S]:** Susceptible, **[I]**: Intermediary.

Table 3: MICs of β-lactams for *S.* Brunei and *S*. Heidelberg producing ESBL

Code	ESBL	CTX	AMX	CFT	CAZ
S. Heidelberg S1	CTX-M-15	>32	>256	>32	48
S. Heidelberg S2	CTX-M-15	>32	>256	>32	48
S. Heidelberg S3	CTX-M-15	>32	>256	>32	48
S. Heidelberg S8	CTX-M-15	>32	>256	>32	48
S. Brunei S10	TEM-188	16	>256	12	32
S. Brunei S12	TEM-4	16	>256	6	64
S. Brunei S22	TEM-4	16	>256	6	64
S. Brunei S9	TEM-4	16	>256	6	64
S. Brunei S15	TEM-48	16	>256	6	64
S. Brunei S16	TEM-48	16	>256	6	64
S. Brunei S18	TEM-48	16	>256	6	64
S. Brunei S20	TEM-48	16	>256	6	64
S. Brunei S21	TEM-48	16	>256	6	64
S. Brunei S23	TEM-48	16	>256	6	64

Legend: **AMX**: amoxicillin, **CTX**: cefotaxime, **CAZ**: ceftazidime, C**FT**: ceftriaxone