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

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

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18 **Abstract**

19 **Objectives:** The purpose of this work was to study the genetic determinants  
20 responsible for extended-spectrum  $\beta$ -lactamase (ESBL) resistance of *Salmonella*  
21 isolated from children during the period 1995 to 2008 at the Hussein Dey hospital in  
22 Algiers (Algeria).

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

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

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

35 **Keywords:**

36 Outbreaks, Children, *Salmonella* Brunei, Heidelberg, Extended-spectrum  $\beta$ -  
37 lactamase, TEM-188

## 38 INTRODUCTION

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

54 To date, more than 340  $\beta$ -lactamases have been described. *Salmonella* have been  
55 found to express a wide variety of ESBL types, including TEM (Ait Mhand *et al.*,  
56 2002), SHV (Hammami *et al.*, 1991), PER (Casin *et al.*, 2003), OXA (Hanson *et al.*,  
57 2002), and CTX-M (Tamang *et al.*, 2011), and, more recently, plasmid-mediated  
58 AmpC type enzymes, such as DHA-1 (Barnaud *et al.*, 1998), CMY-2 (Koeck *et al.*,  
59 1997), and ACC-1 (Rhim-Mahjoubi *et al.*, 2002).

60 In Algeria, ESBLs have been identified in nosocomial isolates of various  
61 *Enterobacteriaceae*, such as *E. coli*, *K. pneumoniae* and *E. cloacae* (Touati *et al.*,  
62 2006; Ibadene *et al.*, 2008; Messai *et al.*, 2008, Ramdani-Bouguessa *et al.*, 2011).  
63 However, only few reports for the presence of these enzymes in *Salmonella* have  
64 been published (Naas *et al.*, 2005; Touati *et al.*, 2008; Ibadene *et al.*, 2009; Naas *et*  
65 *al.*, 2011; Bouzidi *et al.*, 2011).

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

## 69 **METHODS**

### 70 **Bacterial isolates**

71 A collection of 14 non-duplicate amoxicillin-resistant *S. enterica* isolates was  
72 examined. They were obtained from stool samples of children between 1995 and  
73 2008 at the Hussein Dey hospital in Algiers (Algeria). All isolates were biochemically  
74 identified by using the API 20E identification system (BioMérieux, Marcy l'Étoile,  
75 France).

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

### 81 **Susceptibility testing and ESBL detection**

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

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

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

### 98 **β-Lactamases characterization**

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

### 106 **PCR fingerprinting**

107 For enterobacterial repetitive intergenic consensus (ERIC) PCR, whole-cell DNA of  
108 isolates was extracted using the QIAmp DNA mini kit (Qiagen). The primers were  
109 ERIC1 (5'-ATGTAAGCTCCTGGGGATTAC-3') and ERIC2 (5'-  
110 AAGTAAGTACTGGGGTGAGCG-3'). Each 23 µl PCR reaction mixture contained 25  
111 pmol of each primer, 5mmol/L each deoxynucleotide triphosphate (dNTP) and 2.5 U  
112 of Taq polymerase (Qiagen, Courtaboeuf, France) in the manufacturer's provided  
113 buffer. Two microliters of total DNA (about 80 ng) was added to each reaction to  
114 reach the final 25µl PCR reaction volume. The ERIC-PCR parameters were as  
115 follows: initial denaturation at 95°C for 7 min; 30 cycles of denaturation at 92°C for  
116 30 s, annealing at 50°C for 1 min, and extension at 65°C for 8 min; followed by a  
117 final extension at 65°C for 16 min (Cao *et al.*, 2008). PCR amplicons were resolved

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



121 **RESULTS**

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

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

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

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

142 Three isolates of *S. Brunei* (S9, S12 and S22) harbored the *bla*<sub>TEM-4</sub> gene and the  
143 *bla*<sub>TEM-48</sub> gene was found in six isolates of *S. Brunei* (S15, S16, S18, S20, S21 and  
144 S23). The four strains of *S. Heidelberg* were found to produce CTX-M-15.

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

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

157 **Discussion**

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

167 In our study, 10 strains of *S.* Brunei were recovered from infants during the period  
168 1995 to 2008 whereas the 4 *S.* Heidelberg were recovered only in October 2008.  
169 Unfortunately, data of the commonly identified serotypes in Algeria were not  
170 available.

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

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

189 The amino acid substitutions of the sequence of TEM-48 compared to the TEM-1  $\beta$ -  
190 lactamase sequence were Leu21Phe, Gly238Ser, Glu240Lys and Thr265Met. TEM-  
191 48 was first described in *K. pneumoniae* strains in Poland in Poland (Gniadkowski *et*  
192 *al.*, 1998). To our knowledge, no report on TEM-48 isolated from *Salmonella* has  
193 been previously published. Moreover, all isolates of *S. Brunei* producing TEM-48  
194 were resistant to aminoglycosides and sulfonamide.

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

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

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

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

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

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

<b>Target</b>	<b>Primer</b>	<b>Primer sequence</b>	<b>Reference</b>
<i>bla</i> <sub>CTX-M</sub>	CTX-M1-A2	5'-CTTCCAGAATAAGGAATC-3'	Dutour <i>et al.</i> , 2002
	628R	5'-CCTTTCATCCATGTCACCA-3'	Brasme <i>et al.</i> , 2007
	405F	5'-GTGGCGATGAATAAGCTGA-3'	Brasme <i>et al.</i> , 2007
	CTX-M1-B2	5'-CCGTTTCCGCTATTACAA-3'	Dutour <i>et al.</i> , 2002
<i>bla</i> <sub>TEM</sub>	TEM-A	5'-TAAAATTCTTGAAGACG-3'	Chanal <i>et al.</i> , 2000
	TEM-B	5'-TTACCAATGCTTAATCA-3'	
<i>bla</i> <sub>SHV</sub>	SHV-F	5'-ATGCGTTATATTCGCCTGTG-3'	Kojima <i>et al.</i> , 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	GPO	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:** cefpodoxime, **CTX:** cefotaxim, **CAZ:** ceftazidime, **TZP:** piperacillin-tazobactam, **IMP:** imipenem, **FOX:** ceftiofur, **GEN:** gentamicin, **TOB:** tobramycin, **Kan:** kanamycin, **AMK:** amikacin, **SUL:** sulfonamide, **[R]** : Resistant, **[S]**: Susceptible, **[I]**: Intermediary.

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

<b>Code</b>	<b>ESBL</b>	<b>CTX</b>	<b>AMX</b>	<b>CFT</b>	<b>CAZ</b>
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, CFT: ceftriaxone