Emergence of CTX-M-15 type extended-spectrum β -lactamase-producing *Salmonella* spp. in Kuwait and the United Arab Emirates

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Cephalosporins are major antimicrobials used to treat serious *Salmonella* infections. However, their effectiveness is being compromised by the emergence of extended-spectrum β -lactamases (ESBLs). The genetic determinants encoding ESBL in *Salmonella* spp. isolated from patients in Kuwait and United Arab Emirates (UAE) were studied over a 2 year period. Out of a total of 407 isolates, 116 isolates possessed the resistance phenotypes consistent with possible ESBL production. Of these, 69 (59.5%) were ESBL positive. PCR and sequencing were used to determine the genetic determinant(s) responsible for ESBL phenotypes. A total of 14 (12.1%) and 29 (24.6%) isolates were CTX-M-15 ESBL producers and TEM producers, respectively. Ten CTX-M-15 producers carried the insertion sequence *ISEcp1* gene. PFGE analysis revealed identical profiles in 4 of the 13 Kuwaiti strains. This study reports the presence of the *bla*_{CTX-M-15} gene in *Salmonella* spp. and *Salmonella enterica* serotype Typhi from Kuwait and UAE for what is believed to be the first time. This is of great concern as the gene is also found in association with the *ISEcp1* gene, which may easily facilitate its spread. These isolates originated mostly from non-Kuwaiti Arabs rather than from people of Asian origin.

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INTRODUCTION

Salmonella spp. are an important cause of enteric fever and gastroenteritis in humans worldwide. The organisms are transmitted by contaminated food and inadequate hygiene. Typhoidal and serious invasive non-typhoidal *Salmonella* spp. infections are usually treated with antimicrobial agents. Fluoroquinolones and β -lactams are the drugs of choice for invasive salmonella infections. However, resistance has emerged to various classes of antibiotics in many parts of the world with the spread of resistant strains. Both health care-associated outbreaks and community outbreaks have been reported (Arlet *et al.*, 2006).

In *Salmonella*, resistance to cephalosporins is largely due to production of extended-spectrum β -lactamases (ESBLs). Most ESBLs in *Salmonella* are derivatives of TEM and SHV β -lactamase families. Other groups, including PER and CTX-M types, have been described recently (Bonnet, 2004; Bradford, 2001). Also, β -lactamases belonging to either Ambler class B (metallo- β -lactamase) or class A, such as

KPC-2, able to hydrolyse carbapenems, have been described (Miriagou *et al.*, 2003).

CTX-M type ESBLs or cefotaximases, belong to class A β -lactamases and are encoded by *bla*_{CTX-M} genes located in a plasmid or on the chromosome (Rodriguez *et al.*, 2004). Different elements may be involved in the mobilization of *bla*_{CTX-M} genes. Studies with plasmids have confirmed the potential involvement of the insertion sequence *ISEcp1* in the mobility of *bla*_{CTX-M} (Cao *et al.*, 2002). CTX-M enzymes comprise a rapidly growing family of enzymes disseminated in several parts of the world (Bonnet, 2004). A concern is the fact that CTX-M type ESBLs display a level of resistance to cefotaxime (Ctx) and ceftriaxone (Cro) significantly higher than that to ceftazidime (Caz) (Bradford, 2001), although Poirel *et al.* (2002) have described some CTX-Ms, including CTX-M-15, with good activity against Caz. Caz MICs for micro-organisms producing CTX-M type ESBLs are usually within the susceptible range. Therefore, the use of Caz resistance as an indicator of ESBL production may miss ESBL-producing bacteria in the clinical microbiology laboratory. The aim of the study was to find out the burden of CTX-M-15-producing *Salmonella* spp. in Kuwait and United Arab Emirates (UAE), and the problems these strains pose for treatment.

Abbreviations: Amp, ampicillin; Caz, ceftazidime; Cro, ceftriaxone; Ctx, cefotaxime; Cxm, cefuroxime; ESBL, extended-spectrum β -lactamase; UAE, United Arab Emirates.

METHODS

Selection of strains with β -lactam resistance phenotype.

Salmonella spp. isolated from the stool samples of patients with acute diarrhoea were collected between January 2003 and March 2006 from Mubarak Al Kabir Hospital and Infectious Disease Hospital, Kuwait, and Tawam Hospital, Al Ain, UAE. Only single stool isolates for each patient were evaluated. A total of 407 isolates were collected and stored at -80°C until used. These comprised 284 isolates [247 non-typhoidal *Salmonella*, 25 *Salmonella enterica* serotype Typhi (S. Typhi) and 12 *S. enterica* serotype Paratyphi (S. Paratyphi) B and C] from Kuwait and 123 isolates (122 non-typhoidal *Salmonella* and 1 *S. Typhi*) from UAE. They were screened for phenotypes consistent with possible ESBL production using the breakpoint method described by Batchelor *et al.* (2005). Breakpoints used to identify resistance to the β -lactam antibiotics were as follows: ampicillin (Amp) (high-level resistance), $>128\text{ }\mu\text{g ml}^{-1}$; cefuroxime (Cxm), $>16\text{ }\mu\text{g ml}^{-1}$; Ctx, $>1\text{ }\mu\text{g ml}^{-1}$; Cro, $>1\text{ }\mu\text{g ml}^{-1}$; Caz, $>1\text{ }\mu\text{g ml}^{-1}$. Determination of the full MICs for Ctx, Cro, Cxm and Caz for the *bla*_{CTX-M} positive isolates (see below) was carried out using the Etest method (AB Biodisk). The results were collated according to the interpretative criteria recommended by the Clinical and Laboratory Standards Institute (CLSI, 2005).

Detection of ESBL production. ESBL production by selected isolates was detected by the Etest ESBL method using both Caz/Caz combined with clavulanic acid and Ctx/Ctx combined with clavulanic acid strips (AB Biodisk). The tests were carried out and results interpreted as per the manufacturer's instructions. In-house known ESBL-producing *Escherichia coli* and ESBL-negative strains were used as controls.

Detection of resistance genes. DNA template was obtained by using NucleoSpin tissue kit (Macherey-Nagel). ESBL-producing strains were analyzed by PCR to detect the presence of *bla*_{CTX-M}, *bla*_{SHV} and *bla*_{TEM} genes. PCR amplification was performed using published primer pairs (Cao *et al.*, 2002), which are as follows: MA-1 5'-SCS ATG TGC AGY ACC AGT AA-3' and MA-2 5'-CCG CRA TAT GRT TGG TGG TG-3' (for *bla*_{CTX-M}), OS-5 5'-TTA TCT CCC TGT TAG CCA CC-3' and OS-6 5'-GAT TTG CTG ATT TCG CTC GG-3' (for *bla*_{SHV}), and C 5'-TCG GGG AAA TGT GCG CG-3' and D 5'-TGC TTA ATC AGT GAG GCA CC-3' (*bla*_{TEM}). PCR was carried out with the buffer supplied by the manufacturer using 1 μl each primer and 0.25 μl *Taq* polymerase [5 U (μl PE)⁻¹] (GeneAmp Gold PCR reagent kit; Applied Biosystems). The reaction mixture (25 μl) was amplified using the following programs: 5 min 94 $^{\circ}\text{C}$, 30 cycles of 30 s at 94 $^{\circ}\text{C}$, 30 s at 55 $^{\circ}\text{C}$ and 60 s at 72 $^{\circ}\text{C}$, with a final 10 min at 72 $^{\circ}\text{C}$ (for *bla*_{CTX-M}); 5 min 94 $^{\circ}\text{C}$, 30 cycles of 45 s at 94 $^{\circ}\text{C}$, 60 s at 55 $^{\circ}\text{C}$ and 60 s at 72 $^{\circ}\text{C}$, with a final 5 min at 72 $^{\circ}\text{C}$ (for *bla*_{SHV}); and 5 min 94 $^{\circ}\text{C}$, 30 cycles of 30 s at 94 $^{\circ}\text{C}$, 30 s at 60 $^{\circ}\text{C}$ and 60 s at 72 $^{\circ}\text{C}$, with a final 10 min at 72 $^{\circ}\text{C}$ (for *bla*_{TEM}). The amplicon sizes were 550, 797 and 971 bp for *bla*_{CTX-M}, *bla*_{SHV} and *bla*_{TEM}, respectively. The positive control for *bla*_{CTX-M} and *bla*_{TEM} was *Salmonella* strain 971 and the negative control was *Salmonella* C600. The positive control for *bla*_{SHV} was *Salmonella* F1533 and the negative control was C600.

The strains with PCR amplicons positive for *bla*_{CTX-M} were selected for sequencing. PCR products were purified using a NucleoSpin extract II kit (Macherey-Nagel) and sequenced using BigDye terminator cycle sequencing kit version 3.1 on ABI 3100 genetic analyser (Applied Biosystems). The nucleotide sequences were compared with the previously described sequences in the GenBank database using BLAST (<http://www.ncbi.nlm.nih.gov/blast/>).

Detection of *ISEcp1*. The genetic environment of the *bla*_{CTX-M} gene was determined initially by sequencing this short fragment by PCR. Primers, *ISEcp1A* (5'-GCA GGT CTT TTT CTG CTC C-3') and

ISEcp1B (5'-ATT TCC GCA GCA CCG TTT GC-3') (Lartigue *et al.*, 2004) were used. PCR was performed in a 25 μl mixture containing 14 μl sterile Milli Q distilled water, 2.5 μl 10 $\times$ buffer PE, 2.5 μl MgCl_2 , 1 μl primer *ISEcp1A* (5 μM), 1 μl primer *ISEcp1B* (5 μM), 1 μl dNTPs (10 mM PE), 0.3 μl *Taq* polymerase [5 U (μl PE)⁻¹] (GeneAmp Gold PCR reagent kit; Applied Biosystems). The PCR parameters included a denaturation step (5 min at 94 $^{\circ}\text{C}$), which was followed by 35 cycles of amplification (30 s of denaturation at 94 $^{\circ}\text{C}$, 30 s of annealing at 57 $^{\circ}\text{C}$, 60 s of elongation at 72 $^{\circ}\text{C}$) and 7 min at 72 $^{\circ}\text{C}$ final extension. The PCR products were analysed by electrophoresis in a 1% agarose gel. *Salmonella* strain 971 and *Salmonella* strain C600 were used as positive and negative controls, respectively.

Molecular typing. Typing of CTX-M-15 positive strains was performed using PFGE, with *Xba*I-digested DNA separated by electrophoresis in a 1.2% agarose gel as described by the CDC (2002). The strains were compared based on differences in the number and mobility of bands.

RESULTS

Selected bacterial isolates

Based on high-level resistance to Amp and resistance to at least one of the cephalosporins, a total of 116 (28.5%) out of the 407 isolates were further studied. These included 79 strains from Kuwait and 37 from UAE. These isolates were made up of 77 non-typhoidal *Salmonella* spp., 2 *S. Typhi* from Kuwait, and 36 non-typhoidal *Salmonella* spp. and 1 *S. Typhi* from UAE.

Prevalence of ESBL-producing *Salmonella* isolates

As can be seen in ESBL distribution shown in Table 1, of the total of 116 selected isolates, 69 (59.5%) were ESBL-producers; 50 (72.5%) and 19 (27.5%) of these were from Kuwait and UAE, respectively. Of the 50 ESBL-positive isolates in Kuwait, 72% belonged to *S. enterica* serogroups B and C. In contrast, 63.1% ESBL-positive isolates in the UAE belonged to serogroups B and C.

Characterization of the resistance genes

All 116 selected isolates were screened by PCR assays for *bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX-M} genes. Sequencing, followed by BLAST searches, confirmed *bla*_{CTX-M} as *bla*_{CTX-M-15}. Of these 116 isolates, 29 (25%) carried the *bla*_{TEM} gene (21 from Kuwait and 8 from UAE). The *bla*_{CTX-M-15} gene was found in 14 (12.1%) isolates, i.e. 12.1% of total resistant isolates and 20.3% of the ESBL-positive isolates (13 from Kuwait and 1 from UAE), 9 of which also harboured the *bla*_{TEM} gene. However, it is noteworthy that the *bla*_{SHV} gene was not detected in any of the *Salmonella* isolates. Of the 14 isolates positive for the *bla*_{CTX-M-15} gene, 2 (14.3%) were *S. Typhi* and 10 (71.4%) of these isolates also carried the *bla*_{ISEcp1} gene. As shown in Table 2, within the *bla*_{CTX-M-15} positive group, *S. enterica* serotype Typhimurium (*S. Typhimurium*) were the most resistant, followed by *Salmonella* serogroup C and *Salmonella* serogroup B. The two *S. Typhi* did not carry the *bla*_{ISEcp1} gene and

Table 1. Prevalence of ESBL and CTX-M types in *Salmonella* spp. isolated from Kuwait and the UAE

<i>Salmonella</i> spp. (no. from Kuwait/no. from the UAE)	No. (%) of ESBL- & CTX-M-positive strains			
	Kuwait		UAE	
	ESBL	CTX-M	ESBL	CTX-M
<i>S. Typhimurium</i> (19/0)	5 (10)	4 (30.8)	–	–
<i>S. Enteritidis</i> (23/0)	2 (4)	1 (7.7)	–	–
<i>Salmonella</i> group B (60/24)	17 (34)	4 (30.8)	7 (36.8)	0
<i>Salmonella</i> group C (76/30)	19 (38)	2 (15.4)	5 (26.3)	1 (100)
<i>Salmonella</i> group D (35/39)	3 (6)	0	4 (21.1)	0
<i>S. Paratyphi</i> (12/0)	1 (2)	0	–	–
<i>S. Typhi</i> (25/1)	2 (4)	2 (15.4)	1 (5.3)	0
<i>Salmonella</i> spp.* (21/0)	0	0	–	0
Ungroupable (13/30)	1 (2)	0	2 (10.5)	0
Total (284/123)	50 (17.6)	13 (4.6)	19 (15.5)	1 (0.8)

**Salmonella* spp. = *Salmonella* serogroup E (8), *Salmonella* serogroup G (10), *Salmonella* serogroup H (3).

were just as resistant to cefotaxime. The MICs of Caz for all these isolates ranged from 1 µg to 16 µg ml⁻¹ with the two *S. Typhi* being the most resistant.

All the 14 patients infected with *bla*_{CTX-M-15} gene carrying strains were non-Kuwaitis, 10 (71.4 %) of whom were non-Kuwaiti Arab nationals from other Gulf states and the Lebanon. The remaining four patients comprised three Indians and one Pakistani.

Molecular typing

Fig. 1 shows the *Xba*I PFGE fingerprints for the 13 Kuwaiti isolates. Five of the thirteen *bla*_{CTX-M-15} positive isolates

from Kuwait formed a cluster showing identical patterns (see lanes 1, 2, 4, 7 and 9) while the rest presented with different PFGE restriction profiles. The single positive isolate from UAE (U38) had a different restriction profile from the Kuwaiti strains (not shown). As shown in Fig. 1, the *S. Typhi* isolates (K181, K238) had different restriction patterns.

DISCUSSION

In this study, CTX-M and TEM ESBL were the main enzymes found in our isolates. None of the clinical isolates produced SHV ESBL. β -Lactamases are the predominant

Table 2. Characteristics of *bla*_{CTX-M}-producing *Salmonella* isolates from Kuwait and the UAE

Isolate	Serotype/serogroup	Date isolated	MIC of:				Type of ESBL	ISEcpl	Patient nationality
			Amp	Ctx	Caz	Cxm*			
K16	<i>S. Typhimurium</i>	17.06.03	>256	>256	2	>256	CTX-M-15 + TEM	+	NK Arab
K147	<i>S. Typhimurium</i>	11.05.04	>256	>256	3	>256	CTX-M-15 + TEM	+	NK Arab
K152	<i>S. Typhimurium</i>	18.05.04	>256	64	1	128	CTX-M-15 + TEM	+	NK Arab
K180	<i>S. Enteritidis</i>	28.06.04	>256	32	4	64	CTX-M-15 + TEM	–	NK Arab
K181	<i>S. Typhi</i>	28.06.04	>256	16	12	64	CTX-M-15 + TEM	–	Indian
K205	<i>Salmonella</i> spp.	02.08.04	>256	16	4	64	CTX-M-15	+	NK Arab
K235	<i>Salmonella</i> B	09.10.04	>256	32	1.5	128	CTX-M-15	+	Pakistani
K236	<i>Salmonella</i> B	11.10.04	>256	16	0.25	128	CTX-M-15	+	NK Arab
K237	<i>Salmonella</i> B	12.10.04	>256	16	2	>256	CTX-M-15 + TEM	+	NK Arab
K238	<i>S. Typhi</i>	12.10.04	>256	32	16	>256	CTX-M-15 + TEM	–	Indian
K247	<i>Salmonella</i> C2	02.11.04	>256	32	1.5	>256	CTX-M-15 + TEM	+	NK Arab
K270	<i>S. Typhimurium</i>	09.05.05	>256	64	12	>256	CTX-M-15	+	NK Arab
K286	<i>Salmonella</i> B	13.06.05	>256	32	4	128	CTX-M-15	–	NK Arab
U38	<i>Salmonella</i> C	24.11.02	>256	128	2	128	CTX-M-15 + TEM	+	Indian

NK, Non-Kuwaiti.

Fig. 1. PFGE pattern of the 13 CTX-M-15 ESBL-positive *Salmonella* spp. isolated in Kuwait and the ladder size markers. Lanes: 1, *Salmonella* group B (K236); 2, *S. Typhimurium* (K16); 3, *S. Typhimurium* (K147); 4, *S. Typhimurium* (K152); 5, *S. Typhi* (K181); 6, *S. Typhi* (238); 7, *S. Typhimurium* (K270); 8, *Salmonella* group B (K235); 9, *Salmonella* group B (K237); 10, *S. enterica* serotype Enteritidis (*S. Enteritidis*) (K180); 11, *Salmonella* group B (286); 12, *Salmonella* spp. (K205); 13, *Salmonella* group C2 (K247); 14, Pulse marker 50–1000 kb. Strains in lanes 1, 2, 4, 7 and 9 demonstrated PFGE clonal relatedness.

cause of resistance to β -lactam antibiotics in Gram-negative bacteria. Among the Ambler class A ESBL, the TEM, SHV and CTX-M enzymes are the most widely distributed. CTX-M enzymes emerged in the late 1980s, shortly after the introduction of Ctx in clinical practice. Since then, wide dissemination of strains carrying these enzymes has occurred, and led to several outbreaks in hospitals and communities (Arlet *et al.*, 2006). In 2004, *Salmonella* resistant to extended-spectrum cephalosporins were identified in 43 countries encompassing Europe, Latin America, the USA, Taiwan and the Western Pacific (Winokur *et al.*, 2001; Dunne *et al.*, 2000; Li *et al.*, 2005; Su *et al.*, 2005), and its prevalence ranged between 0 and 3.4 %.

This is believed to be the first report of CTX-M type ESBL-producing *Salmonella* in Kuwait and the UAE, and one of the very few reports on the ESBL-producing *Salmonella* in the Middle East (Hammami *et al.*, 1991; Moubareck *et al.*, 2005). The prevalence of ESBL-producing *Salmonella* was about 17 % with a higher proportion (73 %) of these attributable to isolates from Kuwait. This prevalence is much higher than any reported prevalence rates in *Salmonella* spp., such as the prevalence of 1.5 % reported in the Taiwan (Li *et al.*, 2005; Su *et al.*, 2005), 1.9 % in the USA (Dunne *et al.*, 2000), 2.4 % in Latin America (Winokur *et al.*, 2001), 0.8 % in Europe and 3.4 % in the Western Pacific region (Winokur *et al.*, 2001). The reason for the high prevalence of ESBL-producing *Salmonella* in our study may be related to the level of usage of the drug in the community, although we do not have any quantitative data on this.

Our study reports for what is believed to be the first time, the explosive spread of CTX-M-15-producing *Salmonella* isolates and the presence of *bla*_{CTX-M} in *S. Typhi* in the Gulf region, particularly from Kuwait. Until recently, only CTX-M-1, CTX-M-2 and CTX-M-9 have been described in *Salmonella* spp. (Miriagou *et al.*, 2004). There have been reports of outbreaks due to CTX-M-2 ESBL-producing *Salmonella* in Argentina (Rossi *et al.*, 1995; Bauernfeind *et al.*, 1996; Orman *et al.*, 2002) and CTX-M-4- or CTX-M-5-producing *S. Typhimurium* in Russia, Belarus, Hungary and Latvia between 1996 and 1999 (Edelstein *et al.*, 2004; Tassios *et al.*, 1999; Bradford *et al.*, 1998). Outbreaks due to CTX-M-15-producing *Salmonella* strains have not been reported previously. However, sporadic individual infections involving CTX-M-15 ESBL-producing *Salmonella* spp. have been reported in Lebanon and elsewhere (Batchelor *et al.*, 2005; Moubareck *et al.*, 2005; Morris *et al.*, 2006; Kim *et al.*, 2007).

In the past, CTX-M-15 ESBL enzyme has been found almost exclusively in *E. coli* and *Klebsiella* spp., and has been a cause of concern for many clinical microbiologists and infectious diseases experts in the UK and other European countries (Livermore & Hawkey, 2005); it is associated with multiple drug resistance and is spreading rapidly. We have found a sudden and phenomenal increase in the prevalence of *E. coli* and *Klebsiella* clinical isolates producing this enzyme (Poirel *et al.*, 2007). This parallels a sharp rise in the isolation of *E. coli* and *Klebsiella* resistant to Ctx/Cro and many other antibiotics in our teaching hospitals. In a previous study in 1998 (Jamal *et al.*, 1998) on 661 strains of *Salmonella* spp., only 0.3 % of the strains were resistant to Ctx in Kuwait. At present, the resistance rate has gone up fivefold (personal observation). It is conceivable that the *Salmonella* strains in Kuwait and UAE could have acquired the gene encoding this enzyme from *E. coli* and *Klebsiella* in the community, and then it spread among the various species and serotypes of *Salmonella*. This speculation is supported by the fact that the mobile genetic element *ISEcpI*, a single copy insertion sequence responsible for mobilization of *bla* genes and identified upstream of several *bla*_{CTX-M} genes (Walther-Rasmussen & Høiby, 2004), was found in association with about 72 % of our isolates. The only other ESBL found in *Salmonella* in the Middle East is SHV-2 type, which was first reported during a nosocomial outbreak of acute gastroenteritis due to '*Salmonella wien*' in a neonatal intensive care unit in Tunisia (Hammami *et al.*, 1991).

It has been reported from Europe that *bla*_{CTX-M} determinants are often linked to infections in travellers returning to their countries (Batchelor *et al.*, 2005). In 2005, Moubareck and colleagues reported for the first time an ESBL-producing *S. Typhimurium* of CTX-M-15-type in the Lebanon (Moubareck *et al.*, 2005). It was isolated from the stool sample of a 6-year-old boy who did not have a history of travel outside of the Lebanon. Similarly, about 72 % of our CTX-M-15 ESBL-producing isolates were from patients of non-Kuwaiti Arab origin who had no history of

travel in the 6 months preceding their infections. Three of the remaining four patients were of Indian origin and also did not have a history of recent travels. Four of five CTX-M-15 ESBL-producing strains with PFGE clonal relatedness (94 % similarity) were *S. Typhimurium* isolated from unrelated non-Kuwait Arabs, who did not travel out of Kuwait prior to infection, and that were isolated at different periods during the 2 year study. This probably indicates a clonal spread of resistance in this species. The remaining 10 isolates were of diverse origin, suggesting that the spread of the CTX-M-15-mediated resistance may be due to horizontal transfer rather than to spread of a single clone. Although no transfer experiments or plasmid analyses were performed on our isolates, it is reasonable to speculate that these genes are transferable as has been amply demonstrated in the literature (Batchelor *et al.*, 2005; Jin & Ling, 2006).

We did not find any *bla_{SHV}* genes in our isolates. As the members of *bla_{SHV}* group are extremely diverse, we have only included primers able to detect *bla_{SHV}* genes that have been found previously in *Salmonella*. Therefore, we cannot exclude that some of our isolates may contain *bla_{SHV}* genes not previously described in these species.

Many expatriate workers reside in Kuwait and the UAE, and there is a constant movement of people to/from different parts of the world. Usually, we see importation of many infectious diseases from Asia and Africa into the Middle East. However, CTX-M-15-positive *Salmonella* were mostly detected among the Arab population in our study, with the possibility of local origin or origin from other Arab countries. The possibility exists that these *Salmonella* strains could be carried to Asia and Africa, initiating a reverse migration of resistant strains. Already, we have detected the strains in some individuals from the Indian subcontinent.

In conclusion, the finding of the *bla_{CTX-M-15}* gene in *Salmonella* in association with *ISEcp1* is a serious concern, because of the propensity of the latter to facilitate the spread of resistance. This finding is worrying as the treatment of infections involving multiply resistant strains is often difficult. Therefore, measures such as improved food and personal hygiene, and organizing data from patients with similar problems into the same statistical group (patient cohorting), could help to prevent further spread of resistance strains.

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