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16S rRNA Methyltransferases: An Emerging Resistance Mechanism Against Aminoglycosides in *Salmonella*

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1. Introduction

Infections with non-typhoidal *Salmonella* are the second most common cause of bacterial gastroenteritis in developed countries, with the incidence of multidrug-resistant non-typhoidal Salmonella increasing considerably in the last two decades. Non-typhoidal salmonellosis in otherwise healthy individuals usually results in mild, self-limiting diarrhoea, but treatment with an appropriate antimicrobial can be life-saving in vulnerable patient groups such as the elderly, immunocompromised patients or those with underlying disease, and in cases of invasive disease such as *Salmonella* bacteraemia, osteomyelitis and meningitis.

Aminoglycosides are often used in combination with broad-spectrum ß-lactams for the treatment of life-threatening infections due to both Gram-positive and Gram-negative bacteria due to their potent concentration-dependent bactericidal activity and postantibiotic effect, and their ability to act synergistically with many other antimicrobials (Lacy et al., 1998). They have been classified as critically important antimicrobials in human medicine (World Health Organisation, 2007). By binding irreversibly to the highly-conserved aminoacyl (A-site) of the 16S ribosomal subunit they inhibit bacterial protein synthesis, thereby leading to cell death (Kotra et al., 2000). The most common mechanism for resistance to aminoglycosides results from production of aminoglycoside-modifying enzymes (phosphotransferases, nucleotidyltranferases and acetyltransferases), which compromise the binding of the aminoglycoside to the target site. Resistance may also result from reduced intracellular drug uptake and accumulation (likely to be due to changes in membrane permeability) or mutation of ribosomal proteins or rRNA (Mingeot-Leclercq et al., 1999). However, recent years have seen the emergence of several 16S rRNA methyltransferases in clinical isolates of Gram-negative bacteria in Europe, the Far East, and North and South America.

2. Aminoglycoside resistance mediated by 16S rRNA methylation

Aminoglycoside-producing actinomycetes are intrinsically resistant to very high levels (MIC >512 mg/L) of the aminoglycoside they produce due to rRNA methyltransferase enzymes,

but until recently no clinical isolate that was resistant to aminoglycosides as a result of an rRNA methyltransferase had been identified (Davies & Wright, 1997). The first reported 16S rRNA methyltransferase gene *armA* (*a*minoglycoside *r*esistance *m*ethyltransferase) was identified in a multiresistant *Klebsiella pneumoniae* isolated in France (Galimand et al., 2003). A further seven enzymes have been associated with 16S rRNA methylation: *rmtA* (Yokoyama et al., 2003), *rmtB* (Doi et al., 2004), *rmtC* (Wachino et al., 2006a), *rmtD* (now renamed as *rmtD1*) (Doi et al., 2007), *rmtD2* (shares 96.4% amino acid identity with *rmtD1*) (Tijet et al., 2011), *rmtE* (Davis et al., 2010) and *npmA* (Wachino et al., 2007). The deduced amino acid sequences show homology with 16S rRNA methyltransferases produced by *Actinomycetales*, including *Streptomyces* and *Micromonospora* spp. (Fig. 1). However, the degree of identity shared overall between the 16S rRNA methyltransferase genes is less than 30%, which suggests that this is not a recent transfer event.

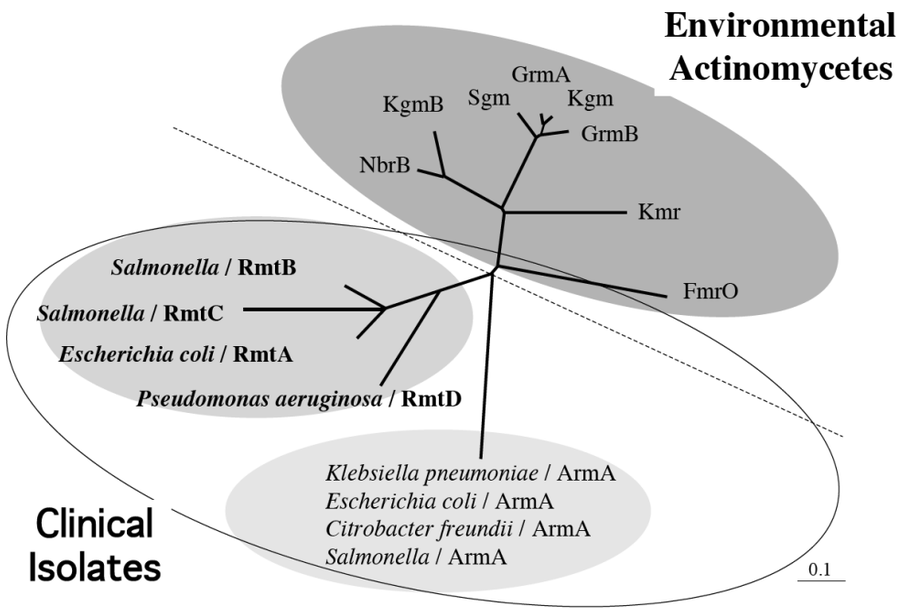

Fig. 1. 16S rRNA methyltransferases conferring high-level resistance to aminoglycosides identified to date.

3. Clinical relevance of 16S rRNA methyltransferases

Unlike the aminoglycoside-modifying enzymes, which vary in their substrate specificity, 16S rRNA methyltransferases *armA* and *rmtA-E* confer high-level resistance to all clinically-important aminoglycosides used for systemic therapy except streptomycin; *npmA* confers high-level resistance mainly to 4,5, disubstituted deoxystreptamines like apramycin. These genes are linked with mobile genetic elements and usually confirmed as located on large conjugative plasmids, allowing potential spread among bacterial populations. In addition, they have been commonly associated with other genes encoding resistance to clinically relevant antimicrobials such as ß-lactams (bla_{SHV}, bla_{CTX-M}, plasmid-mediated AmpC), carbapenems (bla_{SPM-1}, bla_{NDM-1}, bla_{KPC-2}) and fluoroquinolones (plasmid-mediated *qepA*, *aac(6')-Ib-cr* and *qnr* family), thereby allowing potential co-selection and maintenance of

resistance by use of other antimicrobial agents. 16S rRNA methyltransferases have been identified in glucose non-fermentative Gram-negative bacilli and enterobacteria, including *Salmonella enterica,* isolated from clinical, veterinary and food sources.

4. Detection of 16S rRNA methyltransferases

4.1 Phenotypic detection

armA and *rmtA-E* confer resistance to 4,6-disubstituted deoxystreptamines such as amikacin, kanamycin, tobramycin and gentamicin by methylation at position A1405 within the A-site of the 16S rRNA, whilst *npmA* confers panaminoglycoside resistance due to methylation at position A1408 (Liou et al., 2006; Wachino et al., 2007). 16S rRNA methyltransferase producers characteristically demonstrate aminoglycoside MICs ≥256 mg/L and are unique in expressing high-level resistance to the semisynthetic aminoglycoside arbekacin (Doi & Arakawa, 2007). Production of a 16S rRNA methyltransferase should be expected in enterobacteria exhibiting resistance to multiple aminoglycosides according to Clinical and Laboratory Standards Institute (CLSI) guidelines and demonstrating little or no inhibitory zones when using gentamicin, amikacin and arbekacin disks. Although arbekacin may not be readily available Doi & Arakawa (2007) noted that inclusion of an arbekacin disk was preferable as this increased the positive predictive value of this method to ≥90%, compared to only *ca.* 60% when using amikacin alone (Lee et al., 2006). Alternatively, using aminoglycoside MICs ≥256 mg/L as a screen has been reported to have an excellent positive predictive value (Lee et al., 2006; Doi & Arakawa; 2007).

4.2 Molecular detection and characterization

With the exception of *npmA*, phenotypic resistance traits cannot be used to differentiate between the 16S rRNA methyltransferase variants. Instead PCR amplification and comparison of the amplicon sequence to previously reported gene sequences must be used for confirmation. The published literature contains many PCR protocols for detection of these genes (Doi & Arakawa, 2007; Fritsche et al., 2008; Granier et al., 2011).

5. 16S rRNA methyltransferases in *Salmonella enterica*

Although all eight 16S rRNA methyltransferase genes have now been identified in enterobacteria; *armA* and *rmtB* are the most commonly identified and have spread worldwide. The first reported 16S rRNA methyltransferase gene in *Salmonella enterica* was *armA* in *S. enterica* serovar Enteritidis isolated in a hospital in Bulgaria (Galimand et al., 2005). Transfer of high-level aminoglycoside resistance to an Escherichia coli recipient led to transconjugants expressing resistance to 4,6-disubstituted deoxystreptamines due to *armA*, to ß-lactams due to acquisition of $bla_{\text{TEM-1}}$ and $bla_{\text{CTX-M-3}}$, to streptomycin-spectinomycin because of *ant"9*, to sulphonamides because of *sul1* and to trimethoprim because of *dfrXII*. All these resistance genes were located on a *ca.* 90-kb plasmid of incompatibility group IncL/M previously identified in Poland in *Citrobacter freundii* as pCTX-M3 (GenBank accession number AF550415; Gobiewski et al., 2007). Plasmid pCTX-M3 is responsible for the extensive spread of the extended-spectrum ß-lactamase (ESBL) $bla_{\text{CTX-M-3}}$ in enterobacteria in Poland (including within *S. enterica* serovars Typhimurium, Enteritidis, Mbandaka and Oranienburg expressing very high-level resistance to aminoglycosides)

(Baraniak et al., 2002; Gierczynski et al., 2003a; Gierczynski et al., 2003b). The *ant"9*, *sul1*, *dfrXII* and *armA* genes were part of a 16.6-kb composite element flanked by two direct copies of IS*6*, which was designated Tn*1548*. Tn*1548* has been identified on plasmids of other incompatibility groups and in enterobacteria of human and animal origin from several countries, thereby indicating its importance in the dissemination of *armA* (Galimand et al., 2005; González-Zorn et al., 2005a; González-Zorn et al., 2005b).

armA was also identified on a plasmid identical, or very similar to pCTX-M3 in a single isolate of *S. enterica* serovar Virchow among 1,078 non-typhoidal *Salmonella* isolates from patients hospitalized with gastroenteritis in Saint Petersburg, Russia between 2002-2005 (Egorova et al., 2007). The *armA* gene associated with Tn*1548* was also identified in one isolate of *S. enterica* serovar Stanley submitted in 1999 among a collection of 18,261 non-typhoidal *Salmonella* isolated between 1996-2006 at the Centers for Disease Control and Prevention, USA (Folster et al., 2009).

In 2004 an outbreak of diarrhoea occurred in the neonatology unit of the Hospital of Constantine in Algeria. Stool cultures yielded *S. enterica* serovar Senftenberg, which were resistant to amoxicillin, ticarcillin, piperacillin, cefalothin, extended-spectrum cephalosporins, trimethoprim-sulfamethoxazole, amikacin, gentamicin, netilmicin, tobramycin and streptomycin (Naas et al., 2005). Whilst *Salmonella* infections in hospitals are usually food-associated, in this instance spread of infection was likely to be due to horizontal transmission as the commercially prepared milk was fed to other babies that did not become infected. A subsequent screening of 12 representative ESBL-producing serovar Senftenberg isolates isolated from the ward between 1982-2005 that expressed resistance to aminoglycosides was performed (Naas et al., 2009). Only one isolate from 1998 that expressed resistance to all clinically-relevant aminoglycosides was found positive for *armA*; all other isolates were negative for 16S rRNA methyltransferase genes and showed variable levels of resistance to aminoglycosides. As before, *armA* was located within Tn*1548* on a $bla_{\text{CTX-M-3}}$-encoding IncL/M plasmid.

Another outbreak occurred on the neonatology ward of the same hospital between September 2008 to January 2009 (Naas et al., 2011). *S. enterica* serovar Infantis isolates were obtained from 138 patients (mostly from stool cultures but two neonates had positive blood cultures and another had positive gastric fluid). All but two isolates were resistant to all ß-lactams (except cephamycins and carbapenems), kanamycin, netilmicin, tobramycin, amikacin, gentamicin, rifampicin and trimethoprim/sulfamethoxazole. Molecular typing by pulsed-field gel electrophoresis (PFGE) of representative isolates indicated that they were genetically related. The presence of *armA* associated with Tn*1548*, together with $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-1}}$ was confirmed, with the 16S rRNA methyltransferase and ß-lactamase determinants co-located on a 140-kb self-transferable IncL/M plasmid. This suggests that 12 years on, a plasmid similar to that identified in serovar Senftenberg was still present on the ward and may have transferred to serovar Infantis with the $bla_{\text{CTX-M-3}}$ evolving to $bla_{\text{CTX-M-15}}$ via a single amino acid substitution (Asp-240→Gly) that has been associated with increased activity to ceftazidime (Poirel et al., 2002). Other enterobacterial species harbouring both *armA*, and $bla_{\text{CTX-M-3}}$ or $bla_{\text{CTX-M-15}}$ were isolated from Algerian patients transferred to Belgium (Bogaerts et al., 2007), and have been identified in *Klebsiella* spp. in Taiwan and China (Ma et al., 2009; Zhang et al., 2008), indicating that the Infantis plasmid may also have been acquired from a different bacterial species. Implementation and strengthening of

hygiene and infection control measures brought the outbreak under control but was insufficient to prevent further sporadic cases in 2009. These may have occurred after re-hospitalisation of colonised patients or from re-introduction of the strain onto the ward by colonised members of staff.

S. enterica serovars Typhimurium (*n*=13), 4,12:-:1,2 (*n*=1) and Enteritidis (*n*=4) co-harbouring both $bla_{\text{CTX-M-15}}$ and *armA* were identified in another study investigating the prevalence of 16S rRNA methyltransferase genes among ESBL-producing *S. enterica* isolates recovered in Annaba, Algeria between 2008-2009 (Bouzidi et al., 2011). Of the 18 isolates, 13 belonging to serovars Typhimurium (*n*=12) and 4,12:-:1,2 (*n*=1) harboured $bla_{\text{CMY-2}}$ and bla_{TEM} in addition to $bla_{\text{CTX-M-15}}$ and *armA*. PFGE analysis revealed these 12 Typhimurium isolates shared an identical profile, indicating probable spread of an epidemic clone, whilst the four Enteritidis isolates shared three distinct profiles, suggesting horizontal transfer of the resistance determinants.

The continuing spread of pCTX-M3-like plasmids in the *Salmonella* population was highlighted in the recent identification of serovar Gambia strains harbouring a *ca.* 80-kb IncL/M plasmid bearing *armA* and CTX-M-3 together with sulphonamide and trimethoprim resistance determinants in France (Moissenet et al., 2011). The strains isolated from two babies in the intensive care unit of a Paris hospital in 2005 shared identical resistance profiles (resistance to cefotaxime, ceftazidime, gentamicin, tobramycin, amikacin and co-trimoxazole) and indistinguishable PFGE patterns. Given the rarity of serovar Gambia it was concluded that the second baby acquired the strain via cross-infection. Both babies had underlying disease and whilst the first baby was successfully treated with imipenem and ciprofloxacin, the second baby developed further complications and died despite broad-spectrum therapy with imipenem, gentamicin, metronidazole and vancomycin. This was the first report of a fatality associated with non-typhoidal *Salmonella* harbouring a 16S rRNA methyltransferase gene.

6. Enhanced surveillance for 16S rRNA methyltransferases in *Salmonella enterica*

Between March 2006 and December 2009 a retrospective screen to identify 16S rRNA methyltransferase-producing enterobacteria among the strain collections of veterinary, medical and food science research institutes in ten European Union (EU) countries was performed under the auspices of the European-funded MED-VET-NET Network of Excellence. Isolates were selected for further study from nearly one million bacterial isolates from all sources along the food chain, including the environment, food-production animals, food products and humans based on expression of high-level resistance (MIC >256 mg/L) to amikacin or gentamicin. PCR using a harmonized protocol was used to screen selected isolates for the presence of *armA*.

Among 46 *S. enterica* isolates selected from the culture collection of the Health Protection Agency (HPA), UK, five serovar Oranienburg isolates received in 2002 from Poland were positive for *armA* (Hopkins et al., 2007). A *ca.* 90-kb IncL/M conjugative plasmid, which harboured both *armA* and $bla_{\text{CTX-M-3}}$ was isolated, with *armA* borne by Tn*1548* as previously described. Transposon mapping using PCR producing overlapping amplicons along the length of the transposon indicated that the transposon structure was identical to that of pCTX-M3 (Fig. 2).

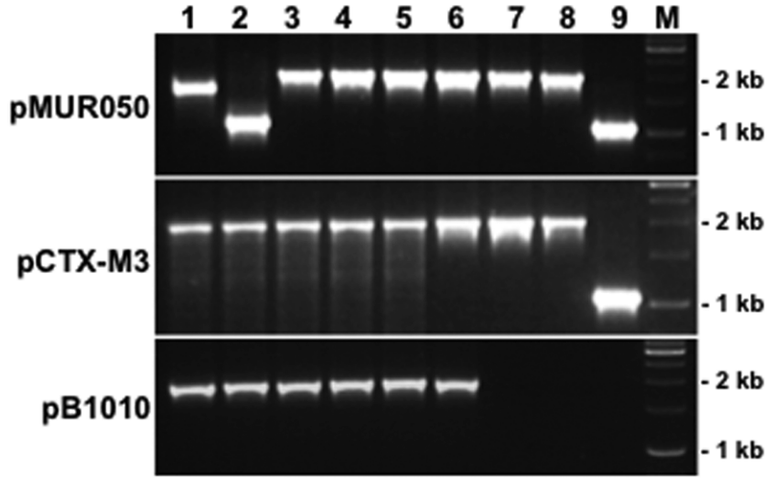

Fig. 2. PCR-mapping of Tn1548, the genetic platform of *armA*. Note that pB1010 has been identified in *Salmonella* from food (Granier et al, 2011).

S. enterica serovar Oranienburg harbouring $bla_{\text{CTX-M-3}}$ and expressing resistance to aminoglycosides has previously been isolated in Poland, where *armA* is estimated to be present in *ca.* 1.6% of human clinical isolates of enterobacteria (Gierczynski et al., 2003a; Gierczynski et al., 2007). These five isolates were part of an outbreak of enteritis associated with an orphanage for infants and children below two years of age in central Poland. The orphanage was reported to provide poor living conditions (due to a high concentration of children) and sanitary standards (i.e. overuse of antimicrobials). Infected children were hospitalised in three separate hospitals in central Poland (Table 1). In total, 52 related isolates were obtained as reported by a local sanitary and epidemiological unit. Until recently this was the only report of involvement of an *armA*-positive *Salmonella* strain in a hospital outbreak (Naas et al., 2009).

Strain identifier	Patient age (months)	Patient symptomatic?	Other information
9/01	7	Y	living at the orphanage; hospitalised in Unit A
11/01	3	Y	living at the orphanage; hospitalised in Unit B
36/01	11	Y	living with parents – no known link to the orphanage
57/01	7	N	living in the orphanage
79/01	11	Y	living with parents – no known link to the orphanage; hospitalised in Unit C.

Table 1. Epidemiological data on *S. enterica* serovar Oranienburg strains

A further 13 isolates of non-typhoidal *Salmonella* were selected based on high-level resistance to amikacin from 81,632 HPA non-typhoidal *Salmonella* isolated between 2004-

2008 (Hopkins et al., 2010). All isolates belonged to serovar Virchow of phage types 25 (*n*=6), 30 (*n*=5) or 31 (*n*=1). PCR was negative for *armA*; however screening for *rmtA*, *rmtB*, *rmtC* and *rmtD* identified *rmtC* (Doi & Arakawa, 2007). RmtC has previously been identified in *Proteus mirabilis* strain ARS68 from an inpatient in Japan (Wachino et al., 2006a) and *P. mirabilis* strain JIE273 from a patient recently returned from India to Australia (Zong et al., 2008), therefore this was the first report of *rmtC* in Europe. Twelve of the 13 strains were isolated from patients between 2005-2008, of which seven had reported recent travel abroad (four to India). *S. enterica* serovar Virchow expressing *rmtC* has also been reported in the United States in a child with a history of travel to India (Folster et al., 2009), suggesting that 16S rRNA methyltransferases may be relatively common in India. The remaining isolate was obtained from frozen produce. This was the first report of a 16S rRNA methyltransferase gene being identified in a bacterial strain isolated from food. Interestingly, a survey in Northern India identified *S. enterica* in 3.6% of vegetable samples; however >30% of isolates were resistant to amikacin (Singh et al., 2007). PFGE revealed the 13 isolates were highly related, showing only one to two band differences; the isolate from frozen produce was indistinguishable from those isolated from patients. Attempts to isolate *rmtC* by conjugation and electroporation experiments were unsuccessful and the gene was eventually discovered located on the chromosome in association with an IS*Ecp1*-like element, which has previously been shown to play a role in the expression and transposition of *rmtC* (Wachino et al., 2006b). The *rmtC* gene was localised on a *ca.* 100-kb non-conjugative plasmid in *P. mirabilis* ARS68, but attempts to transfer *rmtC* from *P. mirabilis* JIE273 to a donor were unsuccessful. This contrasts with *armA* and *rmtB*, which are commonly localised on plasmids (Doi & Arakawa, 2007) and may explain at least in part the higher prevalence and increased dissemination of these genes in comparison to *rmtC*. *rmtC*-positive strains of *S. enterica* serovar Virchow belonging to the same phage types are still being received at the HPA from patients reporting recent return from India, suggesting persistence of this multidrug-resistant clone (Hopkins, unpublished observation).

Further evidence for food being a possible vehicle of infection for bacterial strains harbouring 16S rRNA methyltransferase genes was provided by a recent study reporting the identification of *armA* in a *S. enterica* serovar 4,12:i:- isolate recovered from chicken meat in La Réunion, a French island in the Indian Ocean (Granier et al., 2011). This was the first report of *armA* in a bacterial isolate originating from food. As well as *armA*, the isolate harboured $bla_{\text{CTX-M-3}}$, bla_{TEM}, the *Salmonella* Genomic Island-1 (as previously associated with multidrug-resistant *Salmonella enterica* serovar Typhimurium definitive phage type (DT) 104 (Boyd et al., 2000)), and acquired AmpC ß-lactamase $bla_{\text{CMY-2}}$. Conjugation experiments indicated that *armA*, $bla_{\text{CTX-M-3}}$ and bla_{TEM} were located on the same plasmid. However, further characterisation revealed that, in contrast to previous studies, these genes were located on a *ca.* 110-kb incompatibility group IncP plasmid. IncP plasmids have a broad host range, including *Pseudomonas* spp. and Gram-positive bacteria, therefore association with an IncP plasmid may further broaden the dissemination of *armA*. Transposon mapping using PCR also suggested a deletion event downstream of *armA* and insertion of an IS*26* element, which knocked out genes encoding a macrolide resistance efflux pump and macrolide phosphotransferase gene (Fig. 2 and Fig. 3). Investigation of other *S. enterica* serovar 4,12:i:- isolates recovered from broiler chickens failed to identify any expressing high-level resistance to aminoglycosides, therefore it is likely that this strain was present in the chicken meat sample as a consequence of cross-contamination by a food-handler.

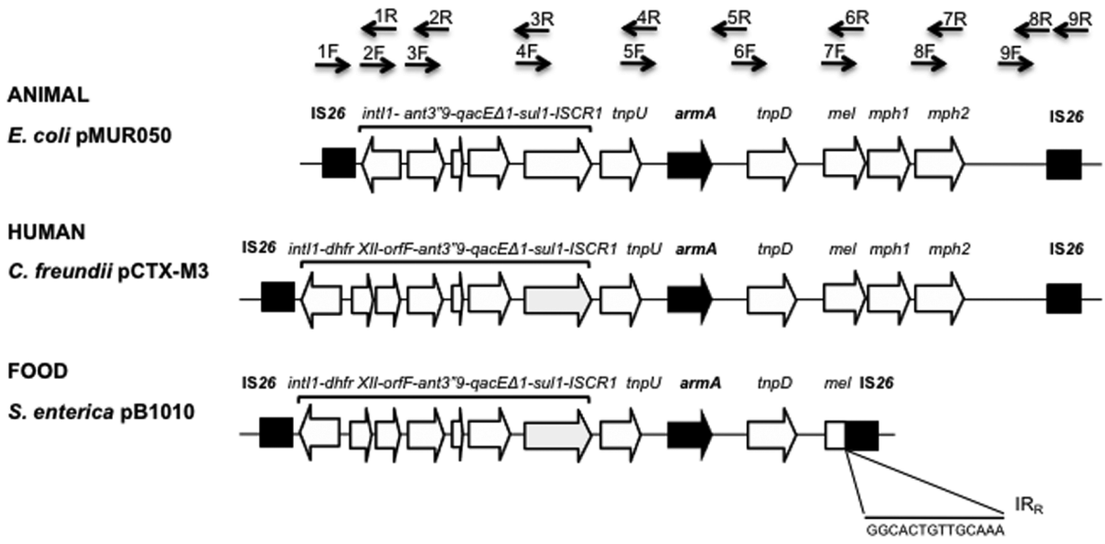

Fig. 3. Schematic representation of the genetic structure of Tn1548 reported from an animal isolate (pMUR050), a human isolate(pCTX-M-3), and a food isolate of Salmonella enterica (pB1010). Blackarrows in the upper part of the panel represent pairs of primers designed for the mapping of Tn1548. (Granier et al., 2011)

7. Conclusion

The worldwide prevalence of *S. enterica* harbouring 16S rRNA methyltransferase genes is very low; however within the EU, Poland appears to represent a major reservoir of these genes. The majority of *S. enterica* isolates have been from nosocomial diarrhoeal outbreaks, with the occurrence of these genes in aminoglycoside-resistant strains from community patients relatively rare. Interestingly, veterinary use of aminoglycosides in food-production animals does not appear to be involved greatly in the spread of 16S rRNA methyltransferase genes as had previously been hypothesized in the literature (González-Zorn et al., 2005a). Food products have recently been identified as a source of *S. enterica* harbouring 16S rRNA methyltransferase genes (Hopkins et al., 2010; Granier et al., 2011), though whether this is an indication of an animal source of these genes, or cross contamination by food handlers has yet to be elucidated. Further evolution and dissemination of these genes may be limited to environments such as hospitals, and countries in which antimicrobial usage is unregulated where a strong selective pressure results from high-level use of a diverse range of aminoglycosides. This is compounded by their common association with other resistance genes, leading to potential co-selection and maintenance of resistance by use of extended-spectrum ß-lactams, carbapenems and fluoroquinolones (Cantón, 2009). Spread of 16S rRNA methyltransferase genes in association with these resistance genes would seriously compromise use of aminoglycosides for treatment of life-threatening infections caused by Gram-negative bacteria such as *S. enterica* and raises the possibility of untreatable *Salmonella* infections. Ongoing surveillance of aminoglycoside resistance in *S. enterica* isolated from humans, animals and food products is therefore crucial to delay the spread of resistance to these classes of antimicrobial agents.

8. References

Baraniak, A., Sadowy, E., Hryniewicz, W. & Gniadkowski, M. (2002). Two Different Extended-Spectrum ß-Lactamases (ESBLs) in One of the First ESBL-Producing Salmonella Isolates in Poland. *Journal of Clinical Microbiology*, Vol.40, No.3, (March 2002), pp.1095-1097, ISSN 0095-1137.

Bogaerts, P., Galimand, M., Bauraing, C., Deplano, A., Vanhoof, R., De Mendonca, R., Robriguez-Villabos, H., Struelens, M. & Glupczynski, Y. (2007). Emergence of ArmA and RmtB Aminoglycoside Resistance 16S rRNA Methylases in Belgium. *The Journal of Antimicrobial Chemotherapy*, Vol.59, No.3, (March 2007), pp.459-464, ISSN 0305-7453.

Bouzidi, N., Aoun, L., Dekhil, M., Granier, S., 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. *The Journal of Antimicrobial Chemotherapy*, published in September edition of JAC (Sep;66(9):2180-1).

Boyd, D., Peters, G., Ng, L. & Mulvey, M. (2000). Partial Characterization of a Genomic Island Associated with the Multidrug Resistance Region of *Salmonella enterica* Typhymurium DT104. *FEMS Microbiology Letters*, Vol.189, No.2, (August 2000), pp.285-291, ISSN 0378-1097.

Cantón, R. (2009). Antibiotic Resistance Genes from the Environment: a Perspective Through Newly Identified Antibiotic Resistance Mechanisms in the Clinical Setting. *Clinical Microbiology and Infection*, Vol.15, Suppl.s1, (January 2009), pp.20-25, ISSN 1469-0691.

Davies, J & Wright, G. (1997). Bacterial Resistance to Aminoglycoside Antibiotics. *Trends in Microbiology*, Vol.5, No.6, (June 1997), pp.234-240, ISSN 0966-842X.

Davis, M., Baker, K., Orfe, L., Shah, D., Besser, T. and Call, D. (2010). Discovery of a Gene Conferring Multiple-Aminoglycoside Resistance in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy*, Vol.54, No.6, (June 2010), pp.2666-2669, ISSN 0066-4804.

Doi, Y., Yokoyama, K., Yamane, K., Wachino, J., Shibata, N., Yagi, T., Shibayama, K., Kato, H. & Arakawa, Y. (2004). Plasmid-Mediated 16S rRNA Methylase in *Serratia marcescens* Conferring High-Level Resistance to Aminoglycosides. *Antimicrobial Agents and Chemotherapy*, Vol.48, No.2, (February 2004), pp.491-496, ISSN 0066-4804.

Doi, Y. & Arakawa, Y. (2007). 16S Ribosomal Methylation: Emerging Resistance Mechanism Against Aminoglycosides. *Clinical Infectious Diseases*, Vol.45, No.1, (January 2007), pp.88-94, ISSN 1058-4838.

Doi, Y., de Oliveira Garcia, D., Adams, J. & Paterson, D. (2007). Coproduction of Novel 16S rRNA Methylase RmtD and Metallo-Beta-Lactamase SPM-1 in a Panresistant *Pseudomonas aeruginosa* Isolate from Brazil. *Antimicrobial Agents and Chemotherapy*, Vol.51, No.3, (March 2007), pp.852-856, ISSN 0066-4804.

Egorova S, Kaftyreva L, Grimont PA, Weill FX. Prevalence and characterization of extended-spectrum cephalosporin-resistant nontyphoidal Salmonella isolates in adults in Saint Petersburg, Russia (2002-2005). Microb Drug Resist. 2007 Summer;13(2):102-7.

Fritsche, T., Castanheira, M., Miller, G., Jones, R. & Armstrong, E. (2008). Detection of Methyltransferases Conferring High-Level Resistance to Aminoglycosides in Enterobacteriaceae from Europe, North America and Latin America. *Antimicrobial Agents and Chemotherapy*, Vol.52, No.5, (May 2008), pp.1843-1845, ISSN 0066-4804.

Folster, J., Rickert, R., Barzilay, E. & Whichard, J. (2009). Identification of the Aminoglycoside Resistance Determinants *armA* and *rmtC* among Non-Typhi *Salmonella* Isolates from Humans in the United States. *Antimicrobial Agents and Chemotherapy*, Vol.53, No.10, (October 2009), pp.4563-4564, ISSN 0066-4804.

Galimand, M., Courvalin, P. & Lambert, T. (2003). Plasmid-Mediated High-Level Resistance to Aminoglycosides in *Enterobacteriaceae* Due to 16S rRNA Methylation. *Antimicrobial Agents and Chemotherapy*, Vol.47, No.8, (August 2003), pp.2565-2571, ISSN 0066-4804.

Galimand, M., Sabtcheva, S., Courvalin, P. & Lambert, T. (2005). Worldwide Disseminated *armA* Aminoglycoside Resistance Methylase Gene is Borne by Composite Transposon Tn*1548*. *Antimicrobial Agents and Chemotherapy*, Vol.49, No.7, (July 2005), pp.2949-2953, ISSN 0066-4804.

Gierczyński, R., Szych, J., Cieślik, A., Rastawicki, W. & Jagielski, M. (2003a). The Occurrence of the First Two CTX-M-3 and TEM-1 Producing Isolates of *Salmonella enterica* Serovar Oranienburg in Poland. *International Journal of Antimicrobial Agents*, Vol.21, No.5, (May 2003), pp.497-499, ISSN 0924-8579.

Gierczyński, R., Szych, J., Rastawicki, W. & Jagielski, M. (2003b). The Molecular Characterisation of the Extended-Spectrum ß-Lactamase (ESBL) Producing Strain of *Salmonella enterica* Serovar Mbandaka Isolated in Poland. *Acta Microbiologica Polonica*, Vol. 52, No.2, pp.183-190, ISSN 0137-1320.

Gierczynski, R., Szych, J., Lawrynowicz-Paciorek, M. & Wardak, S. (2007). Occurrence of the 16S rRNA Methylase *armA* in Human Clinical Isolates of *Enterobacteriaceae* in Poland. In: *3rd Annual Scientific Meeting Abstract Book,* Abstract D&C07, p.23, Med-Vet-Net, Lucca, Italy, June 2007.

Gobiewski, M., Kern-Zdanowicz, I., Zienkiewicz, M., Adamczyk, M., Żylinska, J., Baraniak, A., Gniadkowski, M., Bardowski, J. & Cegłowski, P. (2007). Complete Nucleotide Sequence of the pCTX-M3 Plasmid and Its Involvement in Spread of the Extended-Spectrum ß-Lactamase Gene $bla_{CTX-M-3}$. *Antimicrobial Agents and Chemotherapy*, Vol.51, No.11, (November 2007), pp.3789-3795, ISSN 0066-4804.

González-Zorn, B., Teshager, T., Casa, M., Porrero, M., Moreno, M., Courvalin, P. & Domínguez, L. (2005a). *armA* and Aminoglycoside Resistance in *Escherichia coli*. *Emerging Infectious Diseases*, Vol.11, No.6, (June 2005), pp.954-955, ISSN 1080-6040.

González-Zorn, B., Catalan, A., Escudero, J., Domínguez, L., Teshager, T., Porrero, C. & Moreno, M. (2005b). Genetic Basis for Dissemination of *armA*. *The Journal of Antimicrobial Chemotherapy*, Vol.56, No.3, (September 2005), pp.583-585, ISSN 0305-7453.

Granier, S., Hidalgo, L., San Millan, A., Escudero, J., Gutierrez, B., Brisabois, A. & González-Zorn, B. (2011). ArmA Methyltransferase in a Monophasic *Salmonella enterica* Isolated from Food. *Antimicrobial Agents and Chemotherapy*, published in November edition of AAC (Nov;55(11):5262-6).

Hopkins, K., Catalan, A., Szych, J., Cieslik, A., Gierczyński, R., Threlfall, E. & González-Zorn, B. (2007). Characterisation of *Salmonella* Oranienburg Bearing the 16S rRNA Methylase *armA* from an Enteritis Outbreak in a Polish Hospital. In: *Abstracts of the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy,* Abstract C2-1531, American Society for Microbiology, Chicago, IL, September 2007.

Hopkins, K., Escudero, J., Hidalgo, L & González-Zorn, B. (2010). 16S rRNA Methyltransferase RmtC in *Salmonella enterica* Serovar Virchow. *Emerging Infectious Diseases*, Vol.16, No.4, (April 2010), pp.712-715, ISSN 1080-6040.

Kotra, L., Haddad, J. & Mobashery, S. (2000). Aminoglycosides: Perspectives on Mechanisms of Action and Resistance and Strategies to Counter Resistance. *Antimicrobial Agents and Chemotherapy*, Vol.44, No.12, (December 2000), pp.3249-3256, ISSN 0066-4804.

Lacy, M., Nicolau, D., Nightingale, C. & Quintiliani, R. (1998). The Pharmacodynamics of Aminoglycosides. *Clinical Infectious Diseases*, Vol.27, No.1, (January 1998), pp.23-27, ISSN 1058-4838.

Lee, H., Yong, D., Yum, J., Roh, K., Lee, K., Yamane, K., Arakawa, Y. & Chong, Y. (2006). Dissemination of 16S rRNA Methylase-Mediated Highly Amikacin-Resistant Isolates of *Klebsiella pneumoniae* and *Acinetobacter baumannii* in Korea. *Diagnostic Microbiology and Infectious Disease*, Vol.56, No.3, (November 2006), pp.305-312, ISSN 0732-8893.

Liou, G., Yoshizawa, S., Courvalin, P & Galimand, M. (2006). Aminoglycoside Resistance by ArmA-Mediated Ribosomal Methylation in Human Bacterial Pathogens. *Journal of Molecular Biology*, Vol.359, No.2, (June 2006), pp.358-364, ISSN 0022-2836.

Ma, L., Lin, C., Chen, J., Fung, C., Chang, F., Lai,Y., Lin, J., Siu, L. and the Taiwan Surveillance of Antimicrobial Resistance Project. (2009). Widespread Dissemination of Aminoglycoside Resistance Genes *armA* and *rmtB* in *Klebsiella pneumoniae* Isolates in Taiwan Producing CTX-M-Type Extended-Spectrum β-Lactamases. *Antimicrobial Agents and Chemotherapy*, Vol.53, No.1, (January 2009), pp.104-111, ISSN 0066-4804.

Mingeot-Leclercq, M.-P., Glupczynski, Y. & Tulkens, P. (1999). Aminoglycosides: Activity and Resistance. *Antimicrobial Agents and Chemotherapy*, Vol.43, No.4, (April 1999), pp.727-737, ISSN 0066-4804.

Moissenet, D., Weill, F.-X., Arlet, G., Harrois, D., Girardet, J. & Vu-Thien, H. (2011). *Salmonella enterica* Serotype Gambia with CTX-M-3 and *armA* Resistance Markers: Nosocomial Infections with a Fatal Outcome. *Journal of Clinical Microbiology*, Vol.49, No.4, (April 2011), pp.1676-1678, ISSN 0095-1137.

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 ß-Lactamases from Constantine, Algeria. *The Journal of Antimicrobial Chemotherapy*, Vol.56, No.2, (August 2005), pp.439-440, ISSN 0305-7453.

Naas, T., Bentchouala, C., Lima, S., Lezzar, A., Smati, F., Scheftel, J.-M. & Nordmann, P. (2009). Plasmid-Mediated 16S rRNA Methylases among Extended-Spectrum- ß-Lactamase-Producing *Salmonella enterica* Senftenberg Isolates from Algeria. *The Journal of Antimicrobial Chemotherapy*, Vol.64, No.4, (April 2009), pp.866-867, ISSN 0305-7453.

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 ß-Lactamase in a Neonatology Ward in Constantine, Algeria. *International Journal of Antimicrobial Agents*, Vol.38, No.2, (August 2011), pp.135-139, ISSN 0924-8579.

Poirel, L., Gniadkowski, M. & Nordmann, P. (2002). Biochemical Analysis of the Ceftazidime-Hydrolysing Extended-Spectrum ß-Lactamase CTX-M-15 and of its

StructurallyRrelated ß-Lactamase CTX-M-3. *The Journal of Antimicrobial Chemotherapy*, Vol.50, No.6, (June 2002), pp.1031-1034, ISSN 0305-7453.

Singh, B., Singh, P., Agrawal, S., Teotia, U., Verma, A., Sharma, S., Chandra, M., Babu, N. & Agarwal, R. (2007). Prevalence of Multidrug Resistant *Salmonella* in Coriander, Mint, Carrot, and Radish in Bareilly and Kanpur, Northern India. *Foodborne Pathogens and Disease*, Vol.4, No.2, (Summer 2007), pp.233-240, ISSN 1535-3141.

Tijet, N., Andres, P., Chung, C., Lucero, C., WHONET-Argentina Group, Low, D., Galas, M., Corso, M., Petroni, A. & Melano, R. (2011). *rmtD2*, a New Allele of a 16S rRNA Methylase Gene, Has Been Present in Enterobacteriaceae Isolates from Argentina for More than a Decade. *Antimicrobial Agents and Chemotherapy*, Vol.55, No.2, (February 2011), pp.904-909, ISSN 0066-4804.

Wachino, J., Yamane, K., Shibayama, K., Kurokawa, H., Shibata, N., Suzuki, S., Doi, Y., Kimura, K., Ike, Y. & Arakawa Y. (2006a). Novel Plasmid-Mediated 16S rRNA Methylase, RmtC, Found in a *Proteus mirabilis* Isolate Demonstrating Extraordinary High-Level Resistance Against Various Aminoglycosides. *Antimicrobial Agents and Chemotherapy*, Vol.50, No.1, (January 2006), pp.178-184, ISSN 0066-4804.

Wachino, J., Yamane, K., Kimura, K., Shibata, N., Suzuki, S., Ike, Y. & Arakawa, Y. (2006b). Mode of Transposition and Expression of 16S rRNA Methyltransferase Gene *rmtC* Accompanied by IS*Ecp1*. *Antimicrobial Agents and Chemotherapy*, Vol.50, No.9, (September 2006), pp.3212-3215, ISSN 0066-4804.

Wachino, J., Shibayama, K., Kurokawa, H., Kimura, K., Yamane, K., Suzuki, S., Shibata, N., Ike, Y. & Arakawa, Y. (2007). Novel Plasmid-Mediated 16S rRNA m^1A1408 Methyltransferase, NpmA, Found in a Clinically Isolated *Escherichia coli* Strain Resistant to Structurally Diverse Aminoglycosides. *Antimicrobial Agents and Chemotherapy*, Vol.51, No.12, (December 2007), pp.4401-4409, ISSN 0066-4804.

World Health Organisation (2007). Critically Important Antimicrobials for Human Medicine: Categorization for the Development of Risk Management Strategies to Contain Antimicrobial Resistance Due to Non-Human Antimicrobial Use: Report of the Second WHO Expert Meeting, 29-31 May 2007, Copenhagen, Denmark. 04.07.2011, Available from http://www.who.int/foodborne_disease/resistance/antimicrobials_human.pdf

Yokoyama, K., Doi, Y., Yamane, K., Kurokawa, H., Shibata, N., Shibayama, K., Yagi, T., Kato, H. & Arakawa, Y. (2003). Acquisition of 16S rRNA Methylase Gene in *Pseudomonas aeruginosa*. *The Lancet*, Vol.362, No.9399, (December 2003), pp.1888-1893, ISSN 0140-6736.

Zhang, Y., Zhou, H., Shen, X., Shen, P., Yu, Y. & Li, L. (2008). Plasmid-borne *armA* Methylase Gene, Together with $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1}$, in a *Klebsiella oxytoca* Isolate from China. *Journal of Medical Microbiology*, Vol.57, No.10, (October 2008), pp.1273-1276, ISSN 0022-2615.

Zong, Z., Partridge, S. & Iredell, J. (2008) 16S rRNA Methyltransferase in Australia. *Antimicrobial Agents and Chemotherapy*, Vol.52, No.2, (February 2008), pp.794-794, ISSN 0066-4804.

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