Gram-positive *merA* gene in gram-negative oral and urine bacteria

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Abstract

Clinical mercury resistant (Hg^r) Gram-negative bacteria carrying Gram-positive mercury reductase (*merA*)-like genes were characterized using DNA–DNA hybridization, PCR and sequencing. A PCR assay was developed which discriminated between the *merA* genes related to *Staphylococcus* and those related to the *Bacillus*/*Streptococcus merA* genes by the difference in size of the PCR product. DNA sequence analysis correlated with the PCR assay. The *merA* genes from *Acinetobacter junii*, *Enterobacter cloacae* and *Escherichia coli* were sequenced and shared 98–99% identical nucleotide (nt) and 99.6–100% amino acid identity with the *Staphylococcus aureus* MerA protein. A fourth *merA* gene, from *Pantoeae agglomerans*, was partially sequenced (60%) and had 99% identical nt and 100% amino acid identity with the *Streptococcus oralis* MerA protein. All the Hg^r Gram-negative bacteria transferred their Gram-positive *merA* genes to a Gram-positive *Enterococcus faecalis* recipient with the resulting transconjugants expressing mercury resistance. These Gram-positive *merA* genes join Gram-positive tetracycline resistance and Gram-positive macrolide resistance genes in their association with mobile elements which are able to transfer and express in Gram-negative bacteria.
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1. Introduction

Bacterial resistance to mercury compounds is widespread [1–5]. Bacteria have a number of different genes which confer mercury resistance (Hg^r), though the most common is due to the presence of a mercury reductase (*merA*) gene which reduces reactive ionic Hg^{2+} to volatile monatomic and less toxic elemental Hg^0 [1]. The *merA* gene is usually part of a *mer* operon which contains up to eight additional genes. The *mer* operon has often been linked to antibiotic resistance genes [2,3]. The *mer* genes have been found on chromosomes, integrons, plasmids, and transposons and have been identified in *Enterobacteriaceae* from the pre-antibiotic era (1931–1940) [4,5]. Gram-negative and Gram-positive bacteria, from wide variety of clinical and environmental sources, have similar sets of Hg^r genes in their operons which reduce Hg^{2+} to Hg^0 [2,3].

Mercury resistance can be found on the same elements as antibiotic resistance genes and often have a similar worldwide distribution as antibiotic resistance genes [4–8]. Thirty years ago, it was thought that there were physiological barriers which inhibited gene movement between unrelated Gram-negative bacteria, however in the 1970s the enteric TEM β-lactamase was identified in clinical resistant *Neisseria gonorrhoeae* and *Haemophilus influenzae* [9]. More recently, the hypothesis of a physiological barrier between Gram-positive and Gram-negative bacteria preventing exchange of DNA has been challenged with the realization that the Gram-positive *tet*(M) gene, coding for a

tetracycline resistant ribosomal protection protein, was widely distributed in both Gram-positive and Gram-negative bacteria [9,10], http://faculty.washington.edu/marilynr/. Similarly, the Gram-positive macrolide resistant *mef*(A) efflux gene, which codes for efflux of macrolides, is now commonly found in randomly selected Gram-negative bacteria [11], http://faculty.washington.edu/marilynr/. In this study, we examined whether Hg[r] Gram-negative oral and urine bacteria contain Gram-positive *merA* genes using DNA–DNA hybridization, PCR and DNA sequencing.

2. Materials and methods

2.1. Bacterial isolates

A group of Gram-negative oral and urine isolates collected from healthy children in Lisbon, Portugal, who were participating in a randomized study designed to assess the safety of low-level mercury exposure from dental amalgam restorations and previously characterized for macrolide resistance genes were screened [11]. The isolates were from children who were 8–11 years of age during the recruitment period of February 1997 through April 1998, while isolates were available from cultures obtained between December 1997 and March 1999. The isolates were identified using CHROMagar

orientation media (DRG International Inc, Mountainside, NJ) and standard biochemicals [12]. Isolates were grown on Brain Heart Infusion agar (BHI) (Difco Laboratories, Division of Becton Dickinson & Co., Sparks, MD) supplemented with 100 or 200 μM mercury chloride for 24 h at 36.5 °C before counting colonies (Table 1). We selected 14 mercury resistant (Hg[r]) isolates, representing 10 genera, for further study (Table 1). Hg[r] meant that the isolate could grow on BHI (Brain Heart Infusion) agar (Difco Laboratories) supplemented with 100 or 200 μM mercury chloride. The control Hg[r] Gram-positive *E. faecalis* TX5042b, *E. faecalis* CH116, *S. aureus* 623-3H1, *Streptococcus* sp. 14, *Streptococcus* sp. 56, *S. intermedius* 424, and Gram-negative Hg[r] *E. coli* K12- SK1592(pDU202) were used as controls for the PCR assays. The Gram-positive mercury susceptible (Hg[s]) *E. faecalis* JH2-2 which was fusidic acid, rifampicin, and streptomycin resistant (Fus[r] Rif[r] Str[r]) and the Gram-negative Hg[s] *E. coli* HB101 Fus[r] Rif[r] Str[r] as negative controls for the PCR assays and as recipients in the mating experiments [11].

2.2. Media

BHI agar (Difco Laboratories) unsupplemented or supplemented with 100 or 200 μM mercury chloride was used to verify phenotypic resistance. For matings, BHI plates were supplemented with 100 μM mercury

Table 1
Bacteria in the study

Bacteria	Type of Gram-positive *merA* gene[a]	Gram-negative *merA* gene	Conjugal transferred of *merA* gene into	
			E. faecalis[b]	*E. coli*[c]
Clinical oral isolates				
Acinetobacter junii 329	*Enterococcus/Staphylococcus*	No	+	ND
Citrobacter freundii 16	*Streptococcus*	Yes	+	ND
C. freundii 299	*Streptococcus*	Yes	ND	+
Enterobacter cloacae 19	*Enterococcus/Staphylococcus*	Yes	+	ND
E. coli 11	*Enterococcus/Staphylococcus*	Yes	+	+
Klebsiella sp. 7	*Streptococcus*	Yes	+	ND
Klebsiella sp. 8	*Enterococcus/taphylococcus*	Yes	+	+
K. oxytoca 561	*Streptococcus*	Yes	+	+
Pantoeae agglomerans 323	*Streptococcus*	Yes	+	+
Pseudomonas sp. 333	*Streptococcus*	Yes	+	+
Ralstonia picketti 330	*Streptococcus*	No	+	ND
Clinical urine isolates				
Morganella morganii 6	*Streptococcus*	No	+	ND
Pseudomonas sp. 203	*Enterococcus/Staphylococcus*	Yes	+	+
Stenotrophomonas maltophilia 282	*Enterococcus/Staphylococcus*	Yes	ND	ND
Control isolates				

[a] Based on PCR assay; *Enterococcus/Staphylococcus* type gives a PCR product of 1644 nt; *Bacillus/Streptococcus* type gives a PCR product of 1081 nt.

[b] Frequency of transfer to *E. faecalis* (transconjugants/recipient) ranged between 1.07×10^{-5} and 2.0×10^{-9} all carried Gram-positive *merA* gene; no difference seen between donors that also carried a Gram-negative *merA* gene and those that did not.

[c] Frequency of transfer to *E. coli* (transconjugants/recipient) ranged between 1.8×10^{-5} and 4.7×10^{-9} carried Gram-negative *merA* gene.

chloride plus 500 μg ml^{-1} streptomycin to select for transconjugants, BHI agar supplemented with 500 μg ml^{-1} streptomycin to determine the number of *E. faecalis* recipient or BHI agar supplemented 100 μM mercury chloride to determine the number of donors present as previously described [11,13]. All bacteria were incubated at 36.5 °C.

2.3. Designing primers for detection of Gram-positive merA genes

To develop the Gram-positive *merA* primers for DNA–DNA hybridization, GenBank sequences for the following were used; X99457 from *Exiguobacterium* spp. plasmid, Y09907 from *Bacillus megaterium*, Y10104 from *B. sphaericus* [14], L29436 from *Staphylococcus aureus* plasmid p1258 [15] and a partial sequence from *E. faecalis* CH116 were compared [14]. Two degenerate primers (Hg1 5′ GGA ATT AGG T/CAA AA/T/C/GT A/GTT TCA/T/C 3′ and Hg2 5′ GCA-TAA-ATC/T-ACA/G-TCT-CCA/T-GC 3′) were constructed and shown to hybridize with the Hg^r Gram-positive but not the Hg^s Gram-positive or with any of the Gram-negative laboratory control strains listed in Table 1 (Roberts, M.C., K. Judge, and K. Young. Development of Gram-positive probes for the detection of mercury resistance in oral bacteria. Abstracts of 78th General Session of the International Association for Dental Research, p 621, #3819, Washington DC, April 5–8, 2000). DNA–DNA hybridization assays were done as previously described with ^{32}P-radiolabeled probes [11].

2.4. DNA–DNA hybridization for merA genes

DNA–DNA hybridization of Southern blots, whole cell bacterial dot blots, whole cell DNA dot blots, and/or PCR dot blots were prepared as previously described. These were hybridized with the appropriate ^{32}P-labeled probes as previously described [11]. Isolates, recipients and transconjugants were all tested for the Gram-positive and Gram-negative *merA* genes. Recipients did not carry either *merA* genes.

2.5. PCR detection of merA genes

The PCR assay for detection of the Gram-positive *merA* gene used the two primers (MRAF: 5′ ATG ACT CAA AAT TCA TAT AAA ATA C 3′ and MRAR: 5′ TTA GCC TGC ACA ACA AGA TAA 3′) which produced PCR products from bacillus, enterococcal, staphylococcal and streptococcal *merA* genes (Roberts, M.C., K. Judge, and K. Young. Development of Gram-positive probes for the detection of mercury resistance in oral bacteria. Abstracts of 78th General Session of the International Association for Dental Research, p 621, #3819, Washington DC, April 5–8, 2000).

The size of the PCR product was either 1081 or 1644 nt depending on whether it is a *Streptococcus/Bacillus* or *Enterococcus/Staphylococcus merA*-like gene. The PCR products included the entire *merA* gene and was cloned and then sequenced. The reaction contained 2 U of *Ex Taq* polymerase (Fischer Scientific Co. Houston, TX), 200 μM deoxynucleoside triphosphates, 10× PCR buffer (1.5 mM MgCl$_2$), 100 ng of each primer and 30–40 ng of template DNA with initial 3 min at 96 °C and 35 cycles with 30 s at 96 °C, 1 min at 57 °C, 72 °C for 4 min and 10 min at 72 °C. Positive controls and one negative control were included in each run (Fig. 1).

The PCR assay for detection of the Gram-negative *merA* gene used the two primers (MERA5:5′ACC ATC GTC AGG TAG GGG AAC AA 3′) and (MERA1: 5′ ACC ATC GGC GGC ACC TGC GT 3′) as previously described [15]. Isolates and their transconjugants were screened using DNA–DNA hybridization of whole cell dot blots and/or DNA dot blots for the presence of Gram-negative *merA* genes as previously described [6].

2.6. Sequencing

The PCR products, with the complete *merA* genes, were cloned into the pCR®T7/NT-TOPO® vector (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Primers for the forward and reverse T7
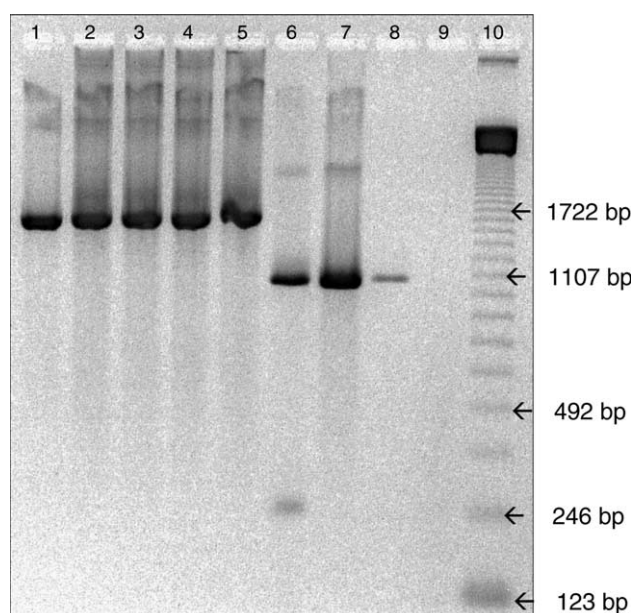

Fig. 1. Agarose gel (1.5%) of PCR products. Lane 1. *E. faecalis* TX5042b *merA* positive [representing the *Enterococcus/Staphylococcus merA* gene group]; lane 2, *E. coli* 11; lane 3, *A. junii* 329; lane 4, *E. cloacae* 19; lane 5. *S. maltophilia* 282; lane 6, *P. agglomerans* 323; lane 7, *R. pickettii* 330; lane 8, *S. intermedius* 424 *merA* positive [representing the *Streptococcus merA* gene group]; lane 9, negative control; lane 10, 123 bp ladder.

was used for sequencing. which was done by the University of Washington, Department of Biochemistry, DNA Sequencing Center. Both DNA and amino acid sequences were compared with other *merA* genes from GenBank. The *A. junii* 329, *E. cloacae* 19, *E. coli* 11, and *P. agglomerans* 323 *merA* genes were assigned GenBank Accession Nos. AY614589, AY614588, AY628209 and AY650024, respectively.

2.7. Mating

Selected Hg[r] clinical isolates were used for conjugation experiments (Table 1). Matings were performed on agar plates using *E. faecalis* JH2-2 as the recipient [11]. The transconjugants were identified as Hg[r] *E. faecalis* Gram-positive cocci which could grow on BHI agar plates (Difco Laboratories) supplemented with 100 μM mercury chloride and 250 μg ml^{-1} streptomycin as previously described. Matings were done as previously

described for transfer of antibiotic resistance genes [11,13]. In other experiments an *E. coli* HB101 recipient was used in the matings as previously described [11]. The type of *merA* gene in the Hg[r] *E. faecalis* and Hg[r] *E. coli* transconjugants were verified using DNA–DNA hybridization, PCR assay and/or partial sequencing.

3. Results

3.1. Detection of merA genes

All 14 Hg[r] clinical isolates examined hybridized with the Hg1 and Hg2 primers suggesting that they carried Gram-positive *merA*-like genes. The Hg[s] Gram-positive and Gram-negative strains neither hybridize to these probes, nor did the Hg[r] Gram-negative control strains (data not shown). In contrast, the Hg[r] *Staphylococcus* sp. and Hg[r] *E. faecalis* gave PCR products of 1644 nt
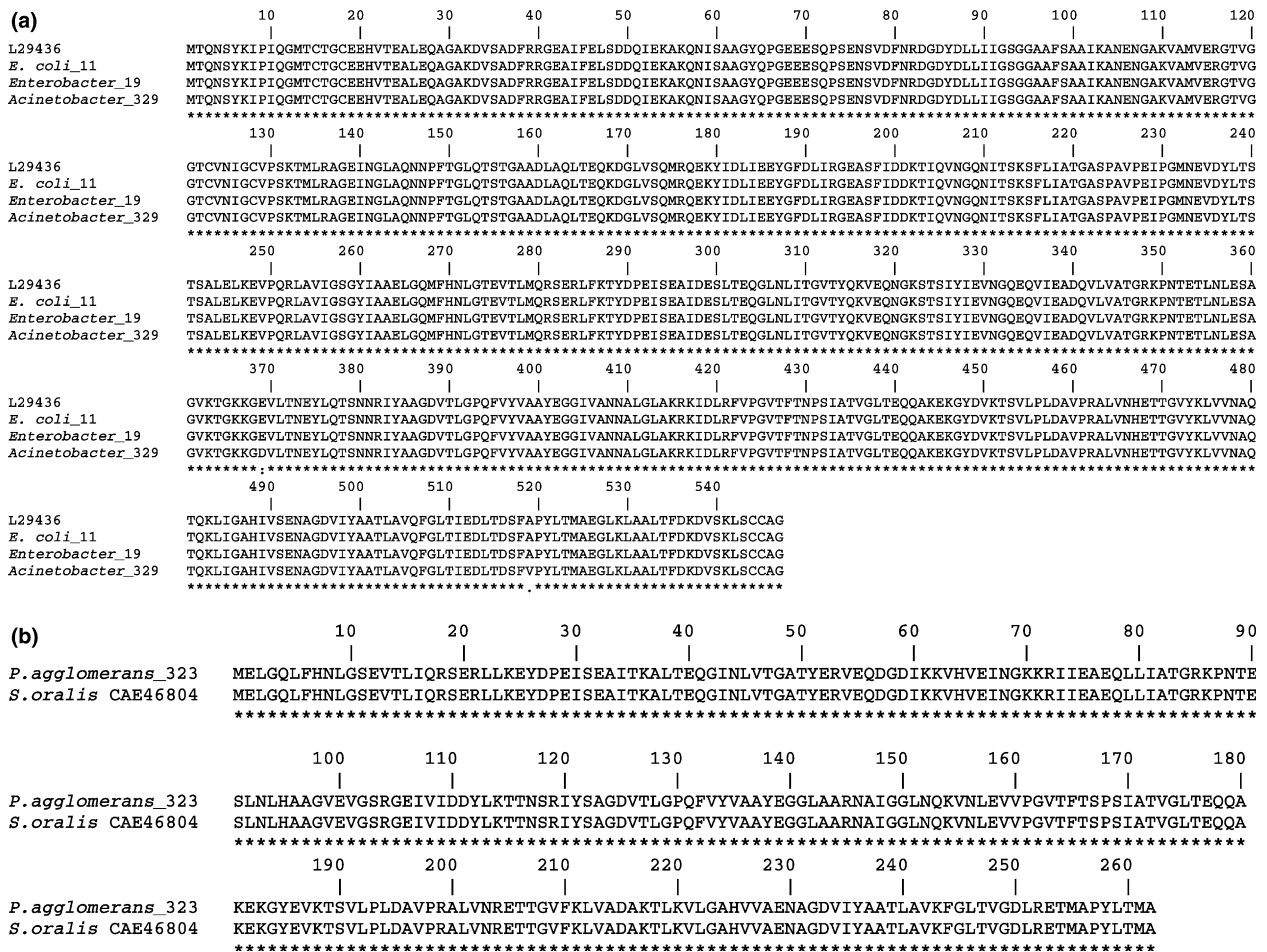

Fig. 2. (a) Multiple alignment of amino acid sequence with the staphylococcal MerA protein (AAA98245). The sequence analysis included the start and stop codons of the *merA* genes and corresponds to 547 amino acids plus the stop codon. A 100% aa homology with *E. coli* 11 and *Enterobacter* 19 MerA protein, while the *Acinetobacter* 329 MerA protein shared 99.6% aa homology with the staphylococcal MerA protein. (b) Amino acid alignment of part of the *P. agglomerans* 323 MerA protein with the corresponding part of the MerA protein of *Streptococcus oralis* (CAE46804) showed 100% homology.

and the Hg^r *Streptococcus* sp. of 1081 nt (Fig. 1, lanes 1 and 8), as expected (Fig. 1, lanes 2–7). All 14 of the Hg^r isolates could be labeled as carrying *Streptococcus merA*-like or *Enterococcus/Staphylococcus merA*-like based on the size of the PCR product produced (Table 1, Fig. 1).

Eleven (79%) of the 14 isolates also carried a Gram-negative *merA* gene, while the remaining three isolates were negative for Gram-negative *merA* gene by DNA–DNA hybridization and by PCR (Table 1). The three isolates that did not carry the Gram-negative *merA* gene, based on DNA–DNA hybridization and PCR assay, included *Acinetobacter junii* 329, *Ralstonia picketti* 330, and *Morganella morganii* 6 (Table 1).

3.2. The Gram-positive merA gene sequences

To verify that the PCR assay correctly grouped the *merA* gene, four isolates representing four genera were selected for sequencing. The PCR amplicons were cloned into pCR®T7/NT-TOPO® vector (Invitrogen) and sequenced from start to stop codon. The *A. junii* 329, *E. cloacae* 19, and the *E. coli* 11 Gram-positive *merA* genes were completely sequenced and the DNA sequences compared with the *S. aureus merA* (L29436) and the amino acids compared (Fig. 2(a)). The *A. junii* 329 sequence shared 98% nt and 99.6% amino acid homology with the *S. aureus merA* gene and MerA protein. Two base pair substitution were identified and included an A to C nt change which resulted in a conserved amino acid substitution from an aspartic acid to glutamic acid at codon 369 and a C to T nt change resulted in a amino acid substitution from alanine to valine at codon 519 (Fig. 2(a)). Three other nucleotide changes within the *A. junii* 329 *merA* sequence did not alter the amino acid sequence. The *E. cloacae* 19 and *E. coli* 11 *merA* sequences shared 99% bp and 100% amino acid homology with the *S. aureus merA* (Fig. 2(a)). Sixty percent of the *merA* gene from *P. agglomerans* 323 was sequenced and shared 99% bp and 100% amino acid homology with the *Streptococcus oralis merA* gene and MerA protein respectively (CAE46804) (Fig. 2(b)). The *merA* genes from *C. freundii* 299, *K. oxytoca* 561, *Klebsiella* sp. 7 and *Klebsiella* sp. 8 were partially sequenced and in each case the *merA* sequences correlated with the size of the PCR product obtained (Table 1).

3.3. Mating experiments

Thirteen of the isolates were used as donors with *E. faecalis* and/or *E. coli* as the recipient (Table 1). Transfer frequencies were low but varied between 1.0×10^{-5} and 2.0×10^{-9}/recipient with the *E. faecalis* recipient (Table 1). The presence of the Gram-positive *merA* genes were verified by DNA–DNA hybridization, PCR assay and/or partial sequencing of the PCR product from the *E.*

faecalis transconjugants. None of the *E. faecalis* transconjugants carried a Gram-negative *merA* genes. There was no consistent differences seen between strains carrying both a Gram-positive and Gram-negative *merA* genes with those only carrying the Gram-positive *merA* gene (data not shown). All the transconjugants were phenotypically Hg^r. Seven of the donors which carried both the Gram-positive and Gram-negative *merA* genes were used in matings with an *E. coli* recipient. Transfer of the Hg^r phenotype varied between 1.8×10^{-5} and 4.7×10^{-9}/recipient and the *E. coli* transconjugants carried the Gram-negative *mer* A gene but not the Gram-positive *merA* gene (Table 1).

4. Discussion

The *merA* genes from Gram-negative and Gram-positive bacteria have been studied for a number of years [1–8,14–19]. However, to our knowledge, this is the first time the Gram-positive *merA* genes have been identified in Gram-negative bacteria, suggesting that gene exchange across major physiological barriers does occur, which is analogous to what has been previously described in Gram-positive tetracycline and macrolide resistant genes [9–11]. The expression of the Hg^r occurred in all 14 isolates, including the three isolates which did not carry the Gram-negative *merA*, suggesting that at least in the three isolates the Gram-positive *merA* genes were expressed. Hg^r *E. faecalis* transconjugants all carried a Gram-positive *merA* gene and no plasmids were found (Table 1). The host range of the Gram-positive *merA* genes suggest that they were associated with conjugative transposons in the Gram-negative donors. Eleven isolates carried both the Gram-positive and Gram-negative *merA* genes and the 10 used in matings were able to transfer their Gram-negative *merA* gene to an *E. coli* recipient but not to the *E. faecalis* recipient. Selective transfer, based on the nature of the recipient, is similar to what we found when looking at transfer of the Gram-negative esterases and phosphorylases, which confer macrolide resistance, from Gram-negative donors to either *E. coli* or *E. faecalis* recipients [11].

The presence of Gram-positive *merA* genes in the Gram-negative population we studied does not seem to be a rare event. In fact, of the 176 original isolates in the previous study [11], 87 (49%) hybridized with the Hg1 and Hg2 primers and included 56% of the oral and 43% of urine isolates. This suggests that the Gram-positive *merA* genes were common among this bacterial population. In addition, two groups of the Gram-positive *merA* genes, those related to the *Enterococcus/Staphylococcus* and a second group related to the *Streptococcus merA* genes, were present in the bacterial population from healthy children. The *Enterococcus/Staphylococcus* could be distinguished from the

Streptococcus merA gene by the size of the PCR products produced (Fig. 1) as well as by their nt differences (Fig. 2(a) and (b)). Both types of *merA* genes were found in oral *Klebsiella* and from both the oral and urine isolates (Table 1).

These 14 isolates also carried a Gram-positive conjugative *mef* (A)–*msr* (D) mobile element which could be transferred to both Gram-positive and Gram-negative recipients [11]. We found co-transfer of these macrolide resistance genes with the Gram-positive *merA* genes. The *mef* (A)–*msr* (D) element appears to be on a composite transposon(s) (authors unpublished observations) and we are currently working to determine if these are physically linked. In addition, seven of the isolates studied also carry the Gram-positive *tet*(M) gene, which is usually associated with a promiscuous transposon of the Tn*916*–Tn*1545* family [10].

The Gram-positive *merA* genes are more widely distributed then previous thought and screening for these genes should be included in future studies of Hg^r Gram-negative bacteria. What role, if any, the Gram-positive *merA* genes and/or their mobile elements, may play in bacterial evolution in Gram-negative bacteria will require further study. It is clear that other ecosystems need to be examined to determine if what was found in these isolates, can be extrapolated to Hg^r Gram-negative bacteria in other populations and environments.

Acknowledgements

References

[1] Narita, M., Chiba, K., Nishizawa, H., Ishii, H., Huang, C.-C., Kawabata, Z., Silver, S. and Endo, G. (2003) Diversity of mercury resistance determinants among *Bacillus* strains isolated from sediment of Minamata Bay. FEMS Microbiol. Lett. 223, 73–82.

[2] Osborn, A.M., Bruce, K.D., Strike, P. and Ritchie, D.A. (1997) Distribution, diversity and evolution of the bacterial merucry resistance (*mer*) operon. FEMS Microbiol. Rev. 19, 239–262.

[3] Barkay, T., Miller, S.M. and Summers, A.O. (2003) Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol. Rev. 27, 355–384.

[4] Essa, A.M.M., Julian, D.J., Kidd, S.P., Brown, N.L. and Hobman, J.L. (2003) Mercury resistance determinants related to Tn*21*, Tn*1696*, and Tn*5053* in Enterobacteria from preantibiotic era. Antimicrob. Agents Chemother. 47, 1115–1119.

[5] Kholodii, G., Mindlin, S., Petrova, M. and Minakhina, S. (2003) Tn*5060* from the Siberian permafrost is most closely related to the ancestor of Tn*21* prior to integron acquistion. FEMS Microbiol. Lett. 226, 251–255.

[6] Liebert, C.A., Hall, R.M. and Summers, A.O. (1999) Transposon Tn*21*, flagship of the floating genome. Microbiol. Mol. Biol. Rev. 63, 507–522.

[7] Yurieva, O., Kholodii, G., Minakhin, L., Gorlenko, Z., Kayaeva, E., Mindlin, S. and Nikiforov, V. (1997) Intercontinental spread of promiscuous mercury-resistance transposons in environmental bacteria. Microbiology 24, 321–329.

[8] Wireman, J., Liebert, C., Smith, T. and Summers, A.O. (1997) Association of mercury resistance with antibiotic resistance in the gram negative fecal bacteria of primates. Appl. Environ. Microbiol. 63, 4494–4503.

[9] Roberts, M.C. (1989) Gene transfer in the urogenital and respiratory tract In: Gene: Transfer in the Environment (Levy, S.B. and Miller, R.V., Eds.), pp. 347–375. McGraw-Hill Publishing Co., New York, NY.

[10] Chopra, I. and Roberts, M.C. (2001) Tetracycline antibiotics: Mode of action, applications, molecular biology and epidemiology of bacterial resistance. Microbiol. Mol. Biol. Rev. 65, 232–260.

[11] Ojo, K.K., Ulep, C.N., Van Kirk, N., Luis, H., Bernardo, M., Leitao, J. and Roberts, M.C. (2004) The *mef* (A) gene predominates among seven macrolide resistant genes identified in 13 Gram-negative genera from healthy Portuguese children. Antimicrob. Agents Chemother. (Sept).

[12] Teixeira, L.M. and Facklam, R.R. (2003) In: Manual of Clinical Microbiology (Murray, P.R., Baron, E.J., Jorgensen, J.H., Pfaller, M.A. and Yolken, R.H., Eds.), 8th Edn, pp. 422–433. ASM Press, Washington, DC.

[13] Agerso, Y., Jensen, L.B., Givskov, M. and Roberts, M.C. (2002) The identification of a tetracycline resistance gene *tet*(M), on a Tn*916*-like transposon, in the *Bacillus cereus* group. FEMS Microbiol. Lett. 214, 251–256.

[14] Bogdanova, E.S., Bass, I.A., Minakhin, L.S., Petrova, M.A., Mindlin, S.Z., Volodin, A.A., Kalyaeva, E.S., Tiedje, J.M., Hobman, J.L., Brown, N.L. and Nikiforov, V.G. (1998) Horizontal spread of *mer* operons among gram-positive bacteria in natural environments. Microbiology 144, 609–620.

[15] Laddaga, R.A., Chu, L., Misra, T.K. and Silver, S. (1987) Nucleotide sequence and expression of the mercurial resistance operon from *Staphylococcus aureus* plasmid p1258. Proc. Natl. Acad. Sci. USA 84, 5106–5110.

[16] Rice, L.B., Marshall, S.H. and Carias, L.L. (1992) Tn*5281*, a conjugative transposon identifiable as a circular form in *Entercoccus faecalis*. J. Bacteriol. 174, 7315–7380.

[17] Liebert, C.A., Wireman, J., Smith, T. and Summers, A.O. (1997) Phylogeny of mercury resistance (*mer*) operons of Gram-negative bacteria isolated from the fecal flora of primates. Appl. Environ. Microbiol. 63, 1066–1076.

[18] Luna, V.A., Cousin Jr., S., Whittington, W.L.H. and Roberts, M.C. (2000) Identification of the conjugative *mef* gene in clinical *Acinetobacter junii* and *Neisseria gonorrhoeae* isolates. Antimicrob. Agents Chemother. 44, 2503–2506.

[19] Luna, V.A., Heiken, M., Judge, K., Uelp, C, Van Kirk, N., Luis, H., Bernardo, M., Leitao, J. and Roberts, M.C. (2002) Distribution of the *mef* (A) gene in Gram-positive bacteria from healthy Portuguese children. Antimicrob. Agents Chemother. 46, 2513–2517.