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Is exposure to mercury a driving force for the carriage of antibiotic resistance genes?

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The mercury resistance gene *merA* has often been found together with antibiotic resistance genes in human commensal *Escherichia coli*. To study this further, we analysed mercury resistance in collections of strains from various populations with different levels of mercury exposure and various levels of antibiotic resistance. The first population lived in France and had no known mercury exposure. The second lived in French Guyana and included a group of Wayampi Amerindians with a known high exposure to mercury. Carriage rates of mercury resistance were assessed by measuring the MIC and by detecting the *merA* gene. Mercury-resistant *E. coli* was found significantly more frequently in the populations that had the highest carriage rates of antibiotic-resistant *E. coli* and in parallel antibiotic resistance was higher in the population living in an environment with a high exposure to mercury, suggesting a possible co-selection. Exposure to mercury might be a specific driving force for the acquisition and maintenance of mobile antibiotic resistance gene carriage in the absence of antibiotic selective pressure.

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INTRODUCTION

Mercury is a heavy metal that occurs naturally in the environment in various forms (Barkay *et al.*, 2003). Methylmercury, one of the most potent neurotoxins known, has adverse effects on the health of animals and humans (Magos & Clarkson, 2006). The consumption of fresh, contaminated fish is the most common means by which humans are exposed to mercury (Barkay *et al.*, 2003). Human activity releases mercury into the air, water and soil (Barkay *et al.*, 2003). The prevalence of mercury-resistant bacteria has been shown to increase in mercury-polluted environments (Barkay & Olson, 1986; Rasmussen & Sorensen, 1998) mainly through selection for acquisition of resistance.

The principal mechanism of bacterial resistance to mercury is the reduction of the reactive ionic form of mercury (Hg^{2+}) to the less-reactive, volatile, elemental form (Hg^0) (Barkay *et al.*, 2003). This reaction is catalysed by the cytosolic flavoenzyme mercuric reductase (MerA) (Fox & Walsh, 1982). Briefly, the *mer* locus consists of genes encoding synthesis of MerA and a transport system which brings Hg^{2+} into the cytoplasm for reduction by MerA. The genes for the entire system are arranged as an operon (the *mer* operon) under both positive and negative genetic regulatory control (Lee *et al.*, 1993), and are often part of the Tn*21*-like transposons, which themselves can carry class 1 integrons, that cannot mobilize without the help of a transposon or an ISCR element, with one or more antibiotic resistance genes (Liebert *et al.*, 1999). It is not clear whether the genetic linkage between antibiotic and mercury resistance is based on selection by an antibiotic pressure, by a mercury pressure, by a combined pressure or due to some other mechanism. Nonetheless, regardless of the presence of dental amalgam fillings that contain mercury, resistance to mercury is widely distributed among bacteria isolated from healthy adults and children, with the presence of mercury resistance genes almost always associated with the concomitant presence of antibiotic resistance genes (Edlund *et al.*, 1996; Liebert *et al.*, 1997;

Pike *et al.*, 2002, 2003; Ready *et al.*, 2003, 2007; Summers *et al.*, 1993; Wireman *et al.*, 1997). This association between antibiotic resistance and heavy metal resistance has also been described in *Staphylococcus aureus*. Indeed, the *S. aureus blaZ*, which encodes penicillin resistance, is part of a transposable element located on a large plasmid, often with additional antimicrobial resistance genes (e.g. gentamicin and erythromycin) (Lowy, 2003). The *blaZ* gene was integrated into a heavy metal resistance plasmid common in the environment and associated, for example, with cadmium resistance (Massidda *et al.*, 2006). Without these plasmid backbones, it is likely that *blaZ* could not have spread among isolates. Whilst heavy metal exposure did not select for penicillin resistance, it is possible that it selected for the resistances available on the plasmid backbones.

METHODS

Bacterial isolates. The isolates studied were commensal *E. coli* obtained from various human populations and stored in our laboratory.

Methods used to isolate and characterize the *E. coli* isolates have been previously reported (Grenet *et al.*, 2004; Skurnik *et al.*, 2008). Briefly, five lactose-positive colonies were randomly selected after culture of faecal samples on Drigalski agar without antibiotics and *E. coli* was identified using API 20E strips (bioMérieux). Methods for determining the antibiotic susceptibility, integron prevalence and phylogenetic groupings of the strains have previously been reported (Aubry-Damon *et al.*, 2004; Grenet *et al.*, 2004; Skurnik *et al.*, 2005, 2008), and were used in the present study. Isolates from the same subjects with identical antibiotic-susceptibility patterns and phylogenetic groups were excluded as replicates (data not shown).

Human subjects. None of the source subjects had taken antibiotics for at least 2 months nor had they been hospitalized for the prior year. They included two distinct populations living in different environments, each composed of two groups, one with a known higher prevalence of antibiotic resistance than the other (Table 1).

The first population was made up of subjects living in mainland France, in a region where environmental mercury exposure was low (2.46 μg g^{-1}) in a study previously presented by the French government (Miquel, 2001). Within this population was a group of 77 pig farmers with high rates of carriage of antibiotic-resistant *E. coli* (280 strains being studied) with no contact known with a farm product that could be a source of mercury exposure, and a second group of 77 bank insurance workers matched with the farmers by age, sex and county of residence, who have been shown to less frequently carry antibiotic-resistant *E. coli* (286 strains being studied) (Table 1) (Aubry-Damon *et al.*, 2004; Skurnik *et al.*, 2005).

The second population was made up of subjects living in French Guyana. Within this population was a group of 56 Wayampi Amerindians living in isolated areas of south Guyana, previously shown to carry high rates of antibiotic-resistant *E. coli* (161 strains being studied) (Skurnik *et al.*, 2008), and who have also been heavily exposed to mercury, probably due to gold mining activities (AFSSET-InVS, 2004; BASAG, 2007). About 15 % of the Wayampi children had a level of mercury in their hair that was higher than the 10 μg g^{-1} recommended by the World Health Organization, and the level of mercury among all of the Wayampi population (7.2 μg g^{-1}) was the highest among individuals tested in French Guyana in 2004 (BASAG, 2007). Of note, all dental care in the Wayampi is administered by intermittent visits of odontologists from the capital city who do not use mercury-containing dental restorations in that population.

The other group was composed of age- and sex-matched French expatriates living in Cayenne City, the capital of French Guyana, located on the northern coast of the territory where the mercury exposure is not significant and who carry lower rates of antibiotic-resistant *E. coli* (122 strains studied) (Table 1) (AFSSET-InVS, 2004; BASAG, 2007; Skurnik *et al.*, 2008).

Mercury resistance. MICs of mercury were determined for each *E. coli* isolate included in the study. Duplicate determinations were made

Table 1. Main characteristics of the studied populations

Characteristic	Result	Comment/reference
Population 1 (France)		
Antibiotic exposure	Pig farmer > bank insurance workers	Exposure through antibiotic therapy (Aubry-Damon *et al.*, 2004)
Mercury exposure	Pig farmer = bank insurance workers	No difference (Miquel, 2001)
Prevalence of antibiotic resistance	Pig farmer > bank insurance workers	Exposure through antibiotic therapy (Aubry-Damon *et al.*, 2004)
Prevalence of mercury resistance	Pig farmer > bank insurance workers	This work
Correlation antibiotic/mercury resistance	Pig farmers and bank insurance workers statistically significant for almost all the tested markers	Exposure through antibiotic therapy (Aubry-Damon *et al.*, 2004)
Population 2 (French Guyana)		
Antibiotic exposure	Wayampi < expatriates	Grenet *et al.* (2004)
Mercury exposure	Wayampi > expatriates	AFSSET-InVS (2004), BASAG (2007)
Prevalence of antibiotic resistance	Wayampi > expatriates	Grenet *et al.* (2004)
Prevalence of mercury resistance	Wayampi > expatriates	This work
Correlation antibiotic/mercury resistance	Wayampi statistically significant only for streptomycin and for trimethoprim; expatriates statistically significant for almost all the tested markers	Exposure through antibiotic therapy (Aubry-Damon *et al.*, 2004)

by the agar dilution method, as described by Ready *et al.* (2003). In brief, cultures were prepared from stock collections stored at −80 °C. Mercuric chloride was added to Müller–Hinton agar at concentrations ranging from 0.125 to 512 μM. Mercury-resistant (NCTC 50581) and mercury-susceptible (8325-4) control strains of *S. aureus* were included in every batch of susceptibility tests as suggested by Ready *et al.* (2003). The mercury-resistant strain *Enterococcus faecium* NCTC 6641 H1 and the mercury-sensitive *E. coli* strain NCTC 10418 were used as additional controls. The four controls were included in duplicate in all of the tests. The positive controls had an MIC of 512 μM. The negative controls had an MIC of 16 μM.

The presence of the *merA* gene was detected in the strains by real-time PCR, using degenerate primers A1s.F (5′-TCCGCAAGTNGCVA-CBGTNGG-3′) and A5-n.r (5′-ACCATCGTCAGRTARGGRAAVA-3′) as described by Vetriani *et al.* (2005). Amplification was in an ABI Prism 7000 SDS thermocycler (Applera) under the following conditions: 95 °C for 10 min followed by 30 cycles of 95 °C for 15 s and 60 °C for 1 min.

Statistical analysis. The chi-square test was used for between-group comparisons, and Fisher's exact test was used when the expected frequencies were under 5. The MacNemar test was used for between-group comparisons of matched observations. A P-value of <0.05 was considered significant. Statistical analyses were performed with Stata software, version 8.0.

RESULTS AND DISCUSSION

A total of 849 *E. coli* strains were tested in duplicate. We found 713 strains with an MIC to mercury of <32 μM and 136 strains with an MIC >64 μM that were regarded as mercury-resistant (Pike *et al.*, 2002). The presence of the *merA* gene was detected in all of the resistant strains and in none of the sensitive ones. These 849 commensal *E. coli* tested came from a total of 266 subjects. Among these subjects, 72 (27 %) were considered carriers of mercury-resistant *E. coli* due to the identification of at least one isolate of mercury-resistant *E. coli.*

The prevalence of carriers of mercury-resistant *E. coli* was significantly higher in the French mainland pig farmers (36.4 %, 28 of 77 subjects carrying 69 resistant strains) than in the French insurance workers (19.5 %, 15 of 77 subjects carrying 30 resistant strains) (P=0.02), and significantly higher in the Wayampi (35.7 %, 20 subjects carrying 21 resistant strains) than in the French expatriates (16.1 %, 9 subjects carrying 16 resistant strains) (P=0.03). Thus, in both the French and the Guyanese populations, carriage rates of mercury-resistant commensal *E. coli* were significantly higher in the groups were there was an overall higher prevalence of antibiotic resistance. This confirmed the well-established link between mercury and antibiotic resistance (Edlund *et al.*, 1996; Liebert *et al.*, 1997; Pike *et al.*, 2002, 2003; Ready *et al.*, 2003, 2007; Summers *et al.*, 1993; Wireman *et al.*, 1997).

The Wayampi have been heavily exposed to mercury (AFSSET-InVS, 2004; BASAG, 2007) and the Tn*21*-like transposons, which often contain the *merA* gene (Liebert *et al.*, 1999), can by chance mobilize an antibiotic containing a class 1 integron onto a plasmid. Thus a putative overall sequence of events for the Wayampi population could be that their mercury exposure has selected *merA* strains and, as these strains were significantly associated with class 1 integrons carrying *aad* and *dhfr* cassettes (Skurnik *et al.*, 2008) when compared to *merA* negative strains (6/16 versus 15/145, P=0.005), this mercury exposure could also have selected streptomycin and trimethoprim resistances.

This hypothesis would explain, in part, why we previously found that antibiotic-resistant *E. coli* was frequently isolated from the Wayampi although their antibiotic use is limited in comparison to residents of mainland France (Grenet *et al.*, 2004). Indeed, the poor hygienic living conditions of the Wayampi may lead to cross-transmission of resistant strains, but our results suggest that this might be only part of the explanation, as environmental exposure to mercury might also play a significant role in selecting out for *merA*-linked antibiotic-resistant genes present within class I integrons. Other authors were recently confronted with similar findings of unexpectedly high levels of acquired antibiotic resistance in commensal *E. coli* isolates from a remote Guaraní Indian community in Bolivia (Pallecchi *et al.*, 2007), combined with very low levels of antibiotic exposure and little contact with the outside world. They suggested that in the absence of direct antibiotic exposure, the maintenance of such a high prevalence of antibiotic resistance might be a selective advantage conferred by genetic determinants linking antibiotic resistance with resistance genes for heavy metal, which in turn might facilitate survival in the environment. It is also possible that mercury resistance plasmids are of a particular type which is more successful than others and thus they could confer a fitness advantage even in the absence of any selective pressure.

Overall, although our study was retrospective and lacked a direct measurement of mercury exposure in the subjects studied, the data that we have presented support the hypothesized link between mercury and antibiotic resistance, which likely should be studied prospectively on a larger scale.

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REFERENCES

AFSSET-InVS (2004). Journée scientifique ''Mercure Guyane''. http://www.afsset.fr/index.php?pageid=738&parentid=424/

Aubry-Damon, H., Grenet, K., Sall-Ndiaye, P., Che, D., Cordeiro, E., Bougnoux, M. E., Rigaud, E., Le Strat, Y., Lemanissier, V. & other authors (2004). Antimicrobial resistance in commensal flora of pig farmers. *Emerg Infect Dis* **10**, 873–879.

Barkay, T. & Olson, B. H. (1986). Phenotypic and genotypic adaptation of aerobic heterotrophic sediment bacterial communities to mercury stress. *Appl Environ Microbiol* **52**, 403–406.

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

BASAG (2007). Le mercure en Guyane. Bulletin d'Alerte et de Surveillance Antilles Guyane. http://www.invs.sante.fr/publications/basag/Basag2007-7.pdf/

Edlund, C., Bjorkman, L., Ekstrand, J., Sandborgh-Englund, G. & Nord, C. E. (1996). Resistance of the normal human microflora to mercury and antimicrobials after exposure to mercury from dental amalgam fillings. *Clin Infect Dis* **22**, 944–950.

Fox, B. & Walsh, C. T. (1982). Mercuric reductase. Purification and characterization of a transposon-encoded flavoprotein containing an oxidation-reduction-active disulfide. *J Biol Chem* **257**, 2498–2503.

Grenet, K., Guillemot, D., Jarlier, V., Moreau, B., Dubourdieu, S., Ruimy, R., Armand-Lefevre, L., Bau, P. & Andremont, A. (2004). Antibacterial resistance, Wayampis Amerindians, French Guyana. *Emerg Infect Dis* **10**, 1150–1153.

Lee, I. W., Livrelli, V., Park, S. J., Totis, P. A. & Summers, A. O. (1993). *In vivo* DNA-protein interactions at the divergent mercury resistance (*mer*) promoters. II. Repressor/activator (MerR)-RNA polymerase interaction with *merOP* mutants. *J Biol Chem* **268**, 2632–2639.

Liebert, C. A., Wireman, J., Smith, T. & 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.

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

Lowy, F. D. (2003). Antimicrobial resistance: the example of *Staphylococcus aureus*. *J Clin Invest* **111**, 1265–1273.

Magos, L. & Clarkson, T. W. (2006). Overview of the clinical toxicity of mercury. *Ann Clin Biochem* **43**, 257–268.

Massidda, O., Mingoia, M., Fadda, D., Whalen, M. B., Montanari, M. P. & Varaldo, P. E. (2006). Analysis of the β-lactamase plasmid of borderline methicillin-susceptible *Staphylococcus aureus*: focus on *bla* complex genes and cadmium resistance determinants *cadD* and c*adX*. *Plasmid* **55**, 114–127.

Miquel, G. (2001). Effets des métaux lourds sur l'environnement et la santé. Office parlementaire d'évaluation des choix scientifiques et technologiques. Rapport 261. http://www.senat.fr/rap/l00-261/l00-261_mono.html

Pallecchi, L., Lucchetti, C., Bartoloni, A., Bartalesi, F., Mantella, A., Gamboa, H., Carattoli, A., Paradisi, F. & Rossolini, G. M. (2007). Population structure and resistance genes in antibiotic-resistant bacteria from a remote community with minimal antibiotic exposure. *Antimicrob Agents Chemother* **51**, 1179–1184.

Pike, R., Lucas, V., Stapleton, P., Gilthorpe, M. S., Roberts, G., Rowbury, R., Richards, H., Mullany, P. & Wilson, M. (2002). Prevalence and antibiotic resistance profile of mercury-resistant oral bacteria from children with and without mercury amalgam fillings. *J Antimicrob Chemother* **49**, 777–783.

Pike, R., Lucas, V., Petrie, A., Roberts, G., Stapleton, P., Rowbury, R., Richards, H., Mullany, P. & Wilson, M. (2003). Effect of restoration of children's teeth with mercury amalgam on the prevalence of mercury- and antibiotic-resistant oral bacteria. *Microb Drug Resist* **9**, 93–97.

Rasmussen, L. D. & Sorensen, S. J. (1998). The effect of longterm exposure to mercury on the bacterial community in marine sediment. *Curr Microbiol* **36**, 291–297.

Ready, D., Qureshi, F., Bedi, R., Mullany, P. & Wilson, M. (2003). Oral bacteria resistant to mercury and to antibiotics are present in children with no previous exposure to amalgam restorative materials. *FEMS Microbiol Lett* **223**, 107–111.

Ready, D., Pratten, J., Mordan, N., Watts, E. & Wilson, M. (2007). The effect of amalgam exposure on mercury- and antibiotic-resistant bacteria. *Int J Antimicrob Agents* **30**, 34–39.

Skurnik, D., Le Menac'h, A., Zurakowski, D., Mazel, D., Courvalin, P., Denamur, E., Andremont, A. & Ruimy, R. (2005). Integron-associated antibiotic resistance and phylogenetic grouping of *Escherichia coli* isolates from healthy subjects free of recent antibiotic exposure. *Antimicrob Agents Chemother* **49**, 3062–3065.

Skurnik, D., Bonnet, D., Bernède-Bauduin, C., Michel, R., Guette, C., Becker, J. M., Balaire, C., Chau, F., Mohler, J. & other authors (2008). Characteristics of human intestinal *Escherichia coli* with changing environments. *Environ Microbiol* **10**, 2132–2137.

Summers, A. O., Wireman, J., Vimy, M. J., Lorscheider, F. L., Marshall, B., Levy, S. B., Bennett, S. & Billard, L. N. (1993). Mercury released from dental "silver" fillings provokes an increase in mercury- and antibiotic-resistant bacteria in oral and intestinal floras of primates. *Antimicrob Agents Chemother* **37**, 825–834.

Vetriani, C., Chew, Y. S., Miller, S. M., Yagi, J., Coombs, J., Lutz, R. A. & Barkay, T. (2005). Mercury adaptation among bacteria from a deep-sea hydrothermal vent. *Appl Environ Microbiol* **71**, 220–226.

Wireman, J., Liebert, C. A., Smith, T. & 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.