to low concentrations of rifampin. To confirm that this effect was a result of persistence rather than generation of resistant mutants, we transferred the colonies growing after transient rifampin exposure of Beijing strain 1585 to a medium containing 8 mg/L rifampin. Their growth was completely inhibited, and molecular analysis did not detect any of the most prevalent rifampin resistance–associated mutants (data not shown).

We believe that these results provide a possible explanation for the otherwise unrealistically high (apparent) mutation frequency reported by de Steenwinkel et al. (2). If these strains are capable of persisting at low concentrations of rifampin, this extended period would provide a window for the generation of mutants during or after exposure. Stress may also play a role; rpoB gene mutants have shown to exhibit a stringent-like response (7), and defective rpoB activity as a result of low-level rifampin exposure could induce a similar response. If rifampin induces a stress response, the situation may be analogous to the high mutation rates seen after quinolone exposure (8).

In summary, our data show that the high apparent M. tuberculosis strain mutation frequency reported by de Steenwinkel et al. (2) may be a result of the higher tolerance to rifampin of some Beijing strains. This tolerance likely results in a specific window of rifampin concentrations that, possibly combined with subsequent error-prone replication/outgrowth, enables the generation and selection of new mutants, rather than the selection of preexisting mutants. When interpreted in the light of our observations, the unexpected results of de Steenwinkel et al. could help explain the association of Beijing genotype strains with drug resistance and relapse (9,10). Drug levels achieved during treatment may be much more critical in preventing the accumulation of rifampin-resistant mutants for these strains than for other genotypes.

This study was financially supported by NanonextNL project 03E.07.

Alice L. den Hertog, Sandra Menting, Dick van Soolingen, and Richard M. Anthony

Author affiliations: KIT Biomedical Research, Royal Tropical Institute (KIT), Amsterdam, the Netherlands (A.L. den Hertog, S. Menting, R.M. Anthony); National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (D. van Soolingen); and Radboud University Medical Center, Nijmegen, the Netherlands (D. van Soolingen)

DOI: http://dx.doi.org/10.3201/eid2011.130560

References

6. den Hertog AL, Visser DW, Ingham CJ, Fey FH, Klatser PR, Anthony RM. Simplified automated image analysis for detection and phenotyping of Mycobacterium tuberculosis on porous supports by monitoring growing microcolonies.


Address for correspondence: Alice L. den Hertog, Royal Tropical Institute (KIT), KIT Biomedical Research, Meibergdreef 39, 1105 AZ Amsterdam, the Netherlands; email: a.d.hertog@kit.nl

Bacteria in Dairy Products in Baggage of Incoming Travelers, Brazil

To the Editor: International air travel can lead to the rapid global dissemination of infectious agents. Unlike products and byproducts of animal origin imported between countries under agreements that legally establish sanitary standards, products introduced into a country illegally or irregularly do not follow specific standards and can come from any source, thereby posing a risk to the health...
status of a country. Animal products transported clandestinely in baggage can contain infectious agents harmful to animal and human health (1–4). We investigated _Brucella_ spp., _Mycobacterium bovis_, and _Mycobacterium avium_ subsp. _paratuberculosis_ (MAP) in dairy products seized from baggage of passengers on flights at the 2 main international airports (Guarulhos Airport, São Paulo, and Galeão Airport, Rio de Janeiro) in Brazil.

During 2010–2011, 12 missions were instigated by the International Agriculture Surveillance (VIGIA-GRO/MAPA) in airports to detect and seize unauthorized dairy products carried by passengers; 195 products were collected from multiple flights from different destinations. Baggage was scanned by using an x-ray machine and, on detection of a product, was opened by the owner in the presence of a federal agriculture inspector. To avoid contamination, the products were not opened and were sent to the designated Ministry of Agriculture, Livestock and Food Supply Laboratory in their original packaging. All seized products were packed according to the International Air Transport Association standards (5) and transported by commercial aviation with official monitoring to the laboratory.

After completing real-time quantitative PCR (Promega, Madison, WI, USA) using TaqMan technology (Life Technologies, Darmstadt, Germany), we extracted DNA directly from the sample (6,7). The technique for the detection of MAP and _eryD_ _Brucella_ (except strain 19 _Brucella abortus_) and also using the region RD4 to detect _M. bovis_ were proposed by Irango et al. (8). To detect _M. bovis_, we used the primers _M. bovis_-88-F (5′-CGC.CTT.CCT.AAC.CAG.AAT.TG-3′), _M. bovis_-88-R (5′-GGA.GAG.CGC.CGT.TGT.AGG-3′) and to detect _Brucella_, we used _Bru-Eri-Taq-92-F_ (5′-GCC.ACA.CTT.TCT.GCA.ATC.TG-3′) and _Bru-Eri-Taq-92-F_ (5′-GCC.GTG.GAT.AAT.GAA.ATC.TGC-3′).

We analyzed 35 containers of dulce de leche, a caramelized milk paste confection, from Argentina (n = 30), Angola (n = 1), and Uruguay (n = 4). We tested all specimens for _Brucella_ spp. and MAP, and 32 for _M. bovis_. We detected MAP in 1 specimen from Argentina and 1 from Uruguay, _Brucella_ spp. in 3 specimens from Argentina and 1 from Uruguay, and _M. bovis_ in 1 specimen from Argentina.

Three containers of liquid milk from the United States were collected and analyzed for the presence of MAP; 2 were analyzed for _M. bovis_ and _Brucella_. _Brucella_ was detected in 1 specimen. Five containers of powdered milk were seized: 2 from Chile, 2 from Angola, and 1 from Portugal. _Brucella_ was detected in 1 container from Chile; _Brucella_ and _M. bovis_ were found in 1 container from Angola. Four containers of yogurt were seized, 1 each from the United States, China, Angola, and South Africa. MAP was detected in those from Angola and South Africa, and the yogurt from South Africa also showed _Brucella._

We analyzed samples from 147 cheeses that were confiscated from baggage owned by travelers from 21 countries, mainly from Italy (24.5%), Portugal (22.4%), and France (14.3%). _M. bovis_ was identified in 18 (17.5%) cheeses collected from Italy, Portugal, Spain, the United States, the Netherlands, Lebanon, Morocco, and Norway. MAP was amplified in specimens from 13 cheeses from Spain, United States, Iraq, Israel, Norway, Peru, France, and Portugal, the last 2 countries showed the highest occurrence. _Brucella_ was detected in 62 of the cheeses analyzed, which originated in Bolivia, Chile, Iraq, Lebanon, and Morocco (n = 1 in each country), Netherlands, Israel, and Norway (n = 2 in each country), Turkey and Spain (n = 3 in each country), United States, France and England (n = 4 in each country), Portugal (n = 10), and Italy (n = 23).

Both _M. bovis_ and _Brucella_ were detected in 13 (8.8%) cheeses (1 each from Spain, Netherlands, Morocco, and Norway; 4 from Portugal, and 5 from Italy). _Brucella_ and MAP were detected in 4 (2.7%) cheeses (Spain, France, Portugal, and Iraq). Co-amplification of the 3 genes (_Brucella_ + MAP + _M. bovis_) occurred in 3 (2%) cheeses (United States, Norway, and Portugal). Among the cheeses analyzed, 84 (57.1%) contained isolates that amplified ≥1 of the genes for the 3 bacteria examined.

Of the 166 dairy products analyzed, _Brucella_ was detected in 70 (42.1%). Cheeses were the most seized products (n = 121) and had the highest number of _Brucella_-positive results (62/121[51.2%]). _Brucella_ was detected in dairy products that originated in Argentina, Spain, France, Iraq, Israel, Lebanon, Portugal, and Turkey; it was detected in 4 (21%) of the 19 cheeses from France and in 3 of the 4 (75%) cheeses that originated in Spain. _M. bovis_ was detected in dulce de leche from Argentina, powdered milk from Chile, and in cheeses from Spain, Netherlands, Italy, Lebanon, Morocco, Norway, and Portugal.

Bacteria can be introduced into a country through contaminated animal products that are brought across borders illegally. The risk may be even greater when these products are carried in passengers’ baggage on international flights because of the growing number of international travelers and the wide range of origins of these passengers. Greater attention should be given to agricultural surveillance at airports to mitigate the risk for introduction of these products.

**Acknowledgment**

We thank CNPq/MAPA/SDA no. 64/2008 for the support.

*Cristiano Barros de Melo,*
*Marcos Eielson Pinheiro de Sá,*
*Antonizete dos Reis Souza,*
*Anapolino Macedo de Oliveira,*
*Pedro Moacyr Pinto Coelho Mota,*
*Paulo Ricardo Campani,*
Janaína Oliveira Luna, Sérigo Cabral Pinto, Fábio Fraga Schwingel, Concepta McManus, and Luíza Seixas

Author affiliations: University of Brasília, Brasilia, Brazil (C.B. de Melo, A.R. Souza, C. McManus, L. Seixas); Ministry of Agriculture, Livestock and Food Supply (MAPA), Brasília, Brazil (M.E.P. de Sa); MAPA, Galeão Airport, Rio de Janeiro, Brazil (P.R. Campani); MAPA, Guarulhos Airport, São Paulo, Brazil (J.O. Luna); MAPA, Confins International Airport, Belo Horizonte/Confins, Brazil (S. Cabral Pinto); MAPA, Brasilia International Airport (BSB), Brasilia, Brazil (F.F. Schwingel); and MAPA, Pedro Leopoldo, Brazil (A.M. de Oliveira, P.M.P.C. Mota)

DOI: http://dx.doi.org/10.3201/eid2011.131422

References

4. de Melo CB, de Sa MEP, Alves FF, McManus C, Aragão LF, Belo BB, et al. Profile of international air passengers in the State of Minas Gerais; Brazil (dissertation) [in Portuguese]. Belo Horizonte, Minas Gerais, Brazil: Federal University of Minas Gerais, 2010.
5. International Air Transport Association.
7. Dias NL. Staphylococcus aureus identification, evaluation of the enterotoxigenic potential and methicillin resistance by the PCR technique in dulce de leche samples in the Sete-Lagoas microregion, in the State of Minas Gerais; Brazil (dissertation) [in Portuguese]. Belo Horizonte, Minas Gerais, Brazil: Federal University of Minas Gerais, 2010.

Address for correspondence: Cristiano Barros de Melo, Universidade de Brasilia (UnB-FAV), Campus Darcy Ribeiro, ICC Sul, Asa Norte, Brasilia, DF 70910-900 Brazil; email: cristianomelo@unb.br

Evidence of Evolving Extraintestinal Enteraggregative Escherichia coli ST38 Clone

To the Editor: Several clones of extended-spectrum β-lactamase (ESBL)–producing extraintestinal pathogenic Escherichia coli (ExPEC) have globally expanded their distribution, including multilocus sequence types (MLSTs) ST38, ST31, ST405, and ST648 (1). ExPEC infections often originate from the patient’s own intestinal flora, although the degree of overlap between diarrheagenic E. coli and ExPEC pathotypes is unclear. Relatively little is known about antimicrobial drug resistance in the most common diarrheagenic E. coli groups, including enteraggregative E. coli (EAEC), and bacterial gastroenteritis is generally managed without use of antimicrobial drugs. The ability of diarrheagenic E. coli to cause extraintestinal infections has been shown in previous studies: a study among children in Nigeria linked EAEC to uropathogenic clonal group A (2), and a study in Brazil showed that EAEC markers were present in 7.1% of the E. coli isolates from urinary tract infections (3). Neither of these studies identified clonal lineages of EAEC specifically associated with extraintestinal infections.

We conducted this study to establish the presence and characteristics of ESBL-producing EAEC in a well-defined collection of ESBL-producing isolates (4). The isolates were from human and animal sources in Germany, the Netherlands, and the United Kingdom. The study was conducted at Public Health England during January–April 2013.

DNA from 359 ESBL isolates (4) was screened for the presence of the EAEC transport regulator gene (aggR), located on the EAEC plasmid, by using a real-time PCR assay and the following primers and probe: AggR_F 5′-CCATTATCGCAATCAGATTAA-3′ AggR_R 5′-CAAGCATCTACTTTGTATTC-3′, AggR_P Cy5-5′AGCCGATACTATTAGACGCCTAAAGGA-BHQ. The amplification parameters were 50°C for 2 min, 95°C for 2 min, and 40 cycles at 95°C for 10 s and at 60°C for 20 s. Isolates positive for aggR were confirmed to be E. coli by using the Omlnog Ge-nIII MicroPlate (Biolog, Hayward, CA, USA). Serotyping was done by using standard methods (5).

The phylogroup was determined for each isolate, and isolates were then assigned to 1 of the 4 major E. coli groups: A, B1, B2, and D (6). A microarray was used to detect ESBL genes, such as blbCTX,M, at the group level, as previously described (4). The antimicrobial drug susceptibilities of EAEC isolates were determined by using the agar incorporation method, as described in the British Society for Antimicrobial Chemotherapy guidelines (7). Virulence factors associated with intestinal and extraintestinal infection...