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Foods confiscated from non-EU flights as a neglected route of potential methicillin-resistant *Staphylococcus aureus* transmission

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ABSTRACT

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in food-producing animals has provoked a great concern in the presence of MRSA in associated foodstuff. In this study, we have assessed for the first time the presence of MRSA in food confiscated from non-EU flights. We performed a search for MRSA among 195 food samples confiscated from passengers on flights from twenty-one non-EU countries in 2012 and 2013. One hundred and seventeen meat samples of diverse animal origin (including antelope, beef, chicken, duck, guinea pig, pork, rodents, and turkey), 75 dairy products (74 cheeses and 1 butter) and 3 eggs were analyzed. All *S. aureus* were studied by pulsed-field gel electrophoresis (PFGE) and antimicrobial susceptibility testing. MRSA isolates were further characterized by multilocus sequence typing (MLST), SCC*mec* typing, and tested for the presence of Panton–Valentine leukocidin (PVL) virulence factors. Overall, 66 food samples were positive for *S. aureus* (33.9%). Six *S. aureus* strains were MRSA (9.1%), all of them in flights from Bolivia (and 5 from the same passenger). Among methicillin-sensitive *S. aureus* (MSSA) (60 out of 66 *S. aureus* strains), 44.1% were resistant to penicillin, 10.2% to tetracycline, 8.5% were resistant to aminoglycosides (amikacin and tobramycin) and 3.4% exhibited the M phenotype. MRSA isolates were sensitive to all non-β-lactam antibiotics tested. *Sma*I-PFGE analysis provided 40 genotypes among the *S. aureus* isolates (three genotypes among the six MRSA). Five MRSA isolates belonged to ST8 and harboured SCC*mec* type IVc as well as PVL genes. One isolate belonged to ST1649, harboured SCC*mec* type IVc and tested negative for the presence of the PVL genes. In conclusion, in this study, we report for the first time the presence of CA-MRSA in food confiscated from non-EU flights: ST8/ST1649-MRSA-IV. These results confirm the illegal entrance of food as a neglected route of transmission as well as the dissemination of successful CA-MRSA lineages among countries via illegal foods. As a result, illegally imported food could play a role in the prevalence and evolution of MRSA clones in the community.

1. Introduction

Staphylococcus aureus is an important cause of food poisoning; it is estimated that an average of 241,148 episodes of domestically acquired foodborne illnesses caused by *S. aureus* occurs in the United States (Scallan et al., 2011). In addition, it is a leading cause of nosocomial invasive infections ranging from mild skin and soft tissue infections to life-threatening diseases such as septicaemia, endocarditic and necrotizing pneumonia (Lowy, 1998). *S. aureus* frequently harbours antibiotic resistance determinants, which complicate treatment and significantly increase the associated costs. Currently, methicillin-resistant *S. aureus* (MRSA) is distributed worldwide and constitutes a major concern in human health because of its complex epidemiology and its ability to acquire novel antibiotic resistance mechanisms.

MRSA was first described as early as 1960, within a year after the inclusion of methicillin in the clinical practice to treat infections caused by the emergence of penicillin-resistant *S. aureus* (Jevons, 1961). Its presence was initially restricted to the clinical environment, but at the end of the past decade, the first cases of MRSA infections in the community were reported, affecting people who exhibited non-typical risk factors of hospital acquisition (Otter and French, 2010). MRSA isolates recovered from the community exhibit particular phenotypic and genotypic features such as distinctive genetic backgrounds, smaller staphylococcal cassette chromosome *mec* (SCC*mec*) types IV or V, a non-multiresistant antibiotic profile and often encode virulence factors such as the Panton–

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Valentine leukocidin (PVL). Consequently, isolates recovered from the community were designated community-associated MRSA (CA-MRSA) to distinguish them from those present in the clinical settings, or hospital-associated MRSA (HA-MRSA). Recently, MRSA belonging to ST398 was observed to colonize pigs and people professionally exposed to pig-farming in several European countries (Voss et al., 2005; Witte et al., 2007). Later studies revealed the presence of that lineage in other food-producing animals, and therefore it was designated as livestock-associated MRSA (LA-MRSA). These findings revealed that companion, livestock and wildlife animals can play a major role as MRSA reservoirs.

The emergence of MRSA in food-producing animals has provoked a great concern in the presence of MRSA in associated foodstuff. The role of food as a vehicle of human MRSA colonization or infection is deemed to be low by the European Food Safety Authority (EFSA). However, foodborne MRSA infections have been formally demonstrated in several occasions (Jones et al., 2002; Kluytmans et al., 1995), and food should not be discarded as a route for transmission of successful LA- and CA-MRSA lineages. In this study, we have evaluated for the first time the presence of MRSA in food confiscated from non-EU flights at an international airport (Bilbao, Spain). This information provides an overview on the rate of MRSA importation via contaminated illegal food, consequently, defining a neglected route of transmission, as well as to reveal which clones are involved.

2. Materials and methods

2.1. Food samples and detection of S. aureus

A total of 195 food samples were confiscated from passengers on flights from twenty-one non-EU countries at the International Bilbao Airport, Spain (www.aeropuertodebilbao.net) from April 2012 to June 2013: 117 meat samples of diverse animal origin (including antelope, beef, chicken, duck, guinea pig, pork, rodents and turkey), 75 dairy products (74 cheeses and 1 butter) and 3 eggs. Samples were confiscated by the Border Authorities at the Border Inspection Post. The origin of the samples was wide: Africa–Equatorial Guinea (7) and Morocco (2), Central and South America–Argentina (8), Bolivia (36), Brazil (13), Colombia (4), Dominican Republic (6), Ecuador (19), Paraguay (6), Peru (29), Puerto Rico (2), and Venezuela (2), Asia–People's Republic of China (31), Nepal (1), and Turkey (5), Eastern Europe–Bosnia and Herzegovina (3), Georgia (4), Moldavia (10), Serbia (3), and Ukraine (3), Oceania Australia (1) and 1 sample from an unknown origin (flight came from Paris, France).

The detection of *S. aureus* was performed following ISO 6888-2 (ISO, 1999). *S. aureus* isolates were further confirmed by real-time PCR as previously described (Trnčíková et al., 2009). A positive colony with the correct morphology in Baird Parker agar was taken for further typing tests (MRSA biotype, antibiotic resistance and genetic typing).

2.2. Screening for the presence of MRSA

The presence of *mec*A and *mec*C in *S. aureus* isolates was tested by multiplex PCR as previously described (Stegger et al., 2012). *mec*C and *mec*A positive controls, as well as non-template controls were included in each run.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the microdilution method following the recommendations and MIC breakpoints of the EUCAST guidelines 2013 v3.1 (www.eucast.org). Susceptibility to the following 20 antimicrobial agents was tested: penicillin, oxacillin, amoxicillin/clavulanate, daptomycin, erythromycin, clindamycin, teicoplanin, vancomycin, ciprofloxacin, levofloxacin, amikacin, gentamicin, tobramycin, mupirocin, rifampin, tetracycline, fusidic acid, fosfomycin, linezolid and cotrimoxazole.

2.4. Characterization of the genetic background

Genetic characterization of *S. aureus* strains was carried out by pulsed-field gel electrophoresis (PFGE) as previously described (McDougal et al., 2003). PFGE patterns were analyzed with Bionumerics v.6.6 (Applied-Maths NV, Sint-Martens-Latem, Belgium) to describe genetic relationships among isolates. Dendograms were constructed using the Dice similarity coefficient and the unweighted pair group mathematical average (UPGMA) clustering algorithm with 1.5% in the tolerance and optimization values. The Simpson index of diversity was calculated to assess the discriminative power of PFGE by using the Comparing Partitions website hosted at darwin.phyloviz.net/ComparingPartitions/index.php?link=Home.

Multilocus sequence typing (MLST) of MRSA strains was performed as described elsewhere (Enright et al., 2000). Allelic profiles obtained by MLST were assigned by comparing the results obtained with data available in the *S. aureus* MLST database hosted at saureus.mlst.net. Information on the MRSAs was submitted to that database.

2.5. Typing and subtyping of the SCCmec element

The genetic structure of the SCC*mec* element was determined by multiplex-PCR as previously described (Kondo et al., 2007). It allows the discrimination of the SCC*mec* types I, II, III, IV, V and VI, as well as the variants IA and IIIA. SCC*mec* IV was further subtyped into 8 different subtypes, from IVa to IVh, by multiplex-PCR as previously described (Milheiriço et al., 2007).

2.6. Detection of Panton–Valentine leukocidin virulence factors

We investigated the presence of the PVL genes (*luk*S-PV and *luk*F-PV) by conventional PCR as described by Lina et al. (1999). Reference strain ATCC 49775 was used as a positive control.

3. Results

3.1. Presence of S. aureus and MRSA in food samples confiscated at the EU point of entrance

Overall, 66 food samples were positive for *S. aureus* (33.9%): 39 cheeses (59.1%), 26 meat products (39.4%) and 1 egg (1.5%). The origin of the positive samples was widespread: Africa, Central and South America, China, and Eastern Europe, but the samples were mainly from Central and South America (Bolivia, 16; Peru, 12; Ecuador, 4; Paraguay, 2; Brazil, 2 and Colombia, 1). The mean *S. aureus* count was 2.82×10^6 CFU/g, ranging from 1.0×10^2 CFU/g in several samples to 9.2×10^7 CFU/g in one cheese from Ecuador (Fig. 1).

Six isolates were MRSA, harbouring the *mec*A resistance determinant (9.1%). The characteristics of the MRSA isolates are detailed in Table 1. Interestingly, all six MRSA were detected in flights from Bolivia. Five MRSA were recovered from the same passenger: four were identified in four different dried meat pieces and another in pork sausages. The sixth MRSA was identified in a cheese sample from a different passenger.

3.2. Antibiotic susceptibility of the S. aureus isolates

Antibiotic susceptibility testing revealed 10 resistance profiles (Table 2). Among the methicillin-sensitive *S. aureus* (MSSA), 44.1% of the isolates were resistant to penicillin, 10.2% to tetracycline, 8.5% were resistant to aminoglycosides (amikacin and tobramycin) and 3.4% exhibited the M-phenotype. MRSA isolates were sensitive to all non-β-lactam antibiotics tested.