International Journal of Food Microbiology 209 (2015) 20–25

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Identification and molecular characterization of pathogenic bacteria in foods confiscated from non-EU flights passengers at one Spanish airport

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ARTICLE INFO

Available online 23 October 2014

Keywords:
Listeria monocytogenes
Salmonella spp.
Campylobacter
Escherichia coli O157:H7
Neglected route of transmission
International flights

ABSTRACT

Two hundred food samples of animal origin confiscated from passengers arriving on flights from non-European countries at the International Airport of Bilbao (Spain) were tested for the presence of four main bacterial foodborne pathogens (*Campylobacter* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp.) during 2012 and 2013. Overall, 20 samples were positive for *L. monocytogenes* (10%) and 11 for *Salmonella* spp. (5.5%), whereas *Campylobacter* spp. and *E. coli* O157:H7 were not detected in any sample. The positive isolates were widely clustered: 14 and 7 different pulsotypes for *L. monocytogenes* and *Salmonella* spp. isolates, respectively. Nine sequence types (ST) were detected for *L. monocytogenes*: ST2 (45%), ST9 (15% isolates), ST8 and ST87 (10%), and ST308, ST37, ST155 and ST378 (5%). The *Salmonella* spp. isolates belonged to seven serovars: monophasic serovar 4,12:d:− (3; 27.3%), Rauform (2; 18.2%), Anatum (2; 18.2%), Oranienburg, Enteritidis, Newport and Typhimurium (1; 9.1% each). Antibiotic resistance among *L. monocytogenes* isolates was high, especially for clindamycin and daptomycin (more than 95% of the isolates). These results indicate that food samples imported by travelers in their personal luggage may harbor the most prevalent *L. monocytogenes* genotypes and *Salmonella* spp. serovars responsible for foodborne outbreaks worldwide. Consequently, international travel can play an important role in the prevalence and dissemination of successful clones of foodborne pathogenic bacteria, and continuous monitoring of international movements is of importance to better understand clonal evolution and emergence and dissemination of successful lineages.

1. Introduction

Foodborne diseases are a global public health threat due to their high prevalence and the associated costs of treatment: up to 30% of the population living in industrialized countries suffer from foodborne diseases every year (Velusamy et al., 2010), and the cost associated is estimated to be 77,700 M$ annually in the USA (Scharff, 2012). The European Food Safety Authority (EFSA) has estimated that over 320,000 human cases are reported each year in the EU, though the real number is likely to be much higher since mild infections are infrequently reported (EFSA, European Food Safety Authority and ECDC, European Centre for Disease Prevention, Control, 2014). Among bacterial foodborne pathogens *Campylobacter*, *Salmonella* spp., verotoxin-producing *Escherichia coli* (VTEC) and *Listeria monocytogenes* are the most frequently reported in the EU, with notification rates per 100,000 population of 214.27, 91.03, 5.67 and 1.64, respectively (EFSA, European Food Safety Authority and ECDC, European Centre for Disease Prevention, Control, 2014).

The potential source of foodborne pathogenic agents is widening, as neglected routes of transmission exist (e.g. cross-border routes). Microbial threats are sent by post or carried in the baggage of travelers arriving from countries outside the EU in the form of personal consignments containing meat, milk or products thereof (Mangili and Gendreau, 2005). As a result, marketing of unauthorized food which has not passed

Abbreviations: CC, clonal complex; CLSI, Clinical and Laboratory Standards Institute; EUCAST, The European Committee on Antimicrobial Susceptibility Testing; MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; RP, resistance profiles; SID, Simpson's Index of Diversity; ST, sequence type; VTEC *Escherichia coli*, verotoxin-producing *Escherichia coli*.

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http://dx.doi.org/10.1016/j.ijfoodmicro.2014.10.016
0168-1605/

the appropriate controls constitutes a neglected route of foodborne pathogen transmission. In this study, we have evaluated for the first time the presence of the main bacterial foodborne pathogens (i.e. *Campylobacter*, *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes*) in food confiscated from non-EU flight passengers from April 2012 to June 2013 at an international airport (Bilbao, Spain); the work was done within the framework of the European research project PROMISE (www.promise-net.eu). This information can provide an overview on the prevalence and the potential risk of foodborne pathogens in illegally imported food. Furthermore this study aims to analyze the routes of pathogenic genotypes being disseminated via international passenger transport.

2. Material and methods

2.1. Food samples

During April 2012 to June 2013, 200 food samples confiscated by the border authorities at the International Bilbao Airport, Spain (www.aeropuertodebilbao.net) from passengers' luggage arriving from 22 non-EU countries were tested. In total, 122 meat samples of diverse animal origin (mainly dried or frozen raw meat samples including antelope, beef, chicken, duck, guinea pig, pork, rodents, and turkey), 75 dairy products (74 cheeses and one butter) and three eggs were included in the confiscated material. The samples were from five continents: Africa [Equatorial Guinea (7) and Morocco (2)], Central and South America [Argentina (9), Bolivia (36), Brazil (13), Colombia (4), Dominican Republic (6), Ecuador (20), Paraguay (6), Peru (29), Puerto Rico (2), and Venezuela (2)], Asia [People's Republic of China (33), Nepal (1), and Turkey (5)], Eastern Europe [Bosnia and Herzegovina (3), Georgia (4), Moldavia (10), Serbia (3), and Ukraine (3)], Oceania–Australia (1)-, and one sample from an unknown origin (flight came from Paris, France).

2.2. Detection of the foodborne pathogens

The detection of thermotolerant *Campylobacter* spp., *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. was performed according to the following international reference methods: ISO 10272–1 (ISO, 2006), ISO 11290–1 (ISO, 1996, 2004), ISO 16654 (ISO, 2001), and ISO 6579 (ISO, 2002). Further confirmation of *L. monocytogenes* and *Salmonella* spp., isolates was performed by specific qPCRs (Rodriguez-Lazaro et al., 2003; Rodríguez-Lázaro et al., 2004; Rodriguez-Lazaro et al., 2005).

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility of the *L. monocytogenes* and *Salmonella* spp. isolates was tested by the microdilution method following the recommendations of the National Committee for Clinical Laboratory Standards. Breakpoints were adapted from the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Antimicrobial susceptibilities of *L. monocytogenes* isolates were tested with 10 antimicrobials: ciprofloxacin, clindamycin, daptomycin, gentamicin, linezolid, penicillin, rifampicin, tetracycline, sulfamethoxazole/trimethoprim, and vancomycin. For *Salmonella* spp. isolates, the antimicrobial susceptibility was tested for the following 29 agents: amikacin, ampicillin, amoxicillin/clavulanate, aztreonam, cefazolin, cefepime, cefotaxime, cefoxitin, ceftazidime, cefuroxime, cephalothin, chloramphenicol, ciprofloxacin, colistin, ertapenem, florfenicol, gentamicin, imipenem, kanamycin, nalidixic acid, piperacillin/tazobactam, streptomycin, sulfamethoxazole, sulfamethoxazole/trimethoprim, tazobactam, tetracycline, tigecycline, tobramycin, and trimethoprim. Isolates were clustered into resistance profiles (RP) according to their susceptibility to the antimicrobials. Isolates exhibiting resistance to three or more antibiotics were considered multiresistant.

2.4. Serotyping of bacterial isolates

Salmonella spp. serotyping was done following the White–Kauffmann–LeMinor scheme (Grimont and Weill, 2007). *L. monocytogenes* serogroups were defined using a multiplex PCR targeting the specific target genes lmo0737, lmo1118, ORF2819, ORF2110 and *Listeria* spp. specific *prs* published by Doumith et al. (2004) and amended by Leclercq et al. (2011) for PCR IVb-VI.

2.5. Genotyping of positive isolates

All isolates were genomically characterized by pulsed-field gel electrophoresis (PFGE) following the standardized protocols from PulseNet (Graves and Swaminathan, 2001; Ribot et al., 2006). 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's index of diversity was calculated to assess the discriminative power of PFGE by using the Comparing Partitions website hosted at http://darwin.phyloviz.net/ComparingPartitions/.

L. monocytogenes isolates were further characterized by multilocus sequence typing (MLST) as previously described (Ragon et al., 2008). Allelic profiles obtained by MLST were assigned by comparing the consensus sequences with the information already published in the Institute Pasteur *L. monocytogenes* MLST database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Lmono.html accessed: 24.04.2014). To define the relationships among strains at the microevolutionary level, an allelic profile-based comparison applying a minimum spanning tree (MST) was performed applying the Institute Pasteur online tool. Clonal complexes (CC) were defined as groups of STs differing by only one housekeeping gene from another member of the group (Ragon et al., 2008). The adjusted Wallace coefficient for quantification of the agreement between PFGE typing and MLST was calculated on the Comparing Partitions website.

3. Results

3.1. Presence of foodborne pathogens in the tested food samples

Twenty samples were positive for *L. monocytogenes* (10%, 20/200), including five from cheese, thirteen from meat, and two from poultry samples. Eleven samples were positive for *Salmonella* spp., including five cheese, four meat samples and two poultry samples. Only one sample (a cheese from Bolivia) was co-contaminated by both pathogens. However, *Campylobacter* spp. and *E. coli* O157:H7 were not detected in any sample. Eleven *L. monocytogenes* isolates (55%) were related to genetic lineage I (PCR-serogroup 4b, 4d, 4e; 1/2b, 3b), and nine to genetic lineage II (PCR-serogroup 1/2a, 3a; 1/2c, 3c). *Salmonella* spp. isolates belonged to seven serovars: monophasic serovar 4,12:d:− (27.3%), Rauform (18.2%), Anatum (18.2%), Oranienburg, Enteritidis, Newport and Typhimurium (9.1% each).

3.2. Antimicrobial susceptibility

All *L. monocytogenes* isolates showed antimicrobial resistance to at least one antibiotic and were assigned to four RP (Table 1): all isolates were resistant to clindamycin, 19 to daptomycin (95%), two to tetracycline (10%) and one to ciprofloxacin (5%). Three RPs were detected for the 11 *Salmonella* spp. isolates (Table 1): 3 isolates were resistant to ciprofloxacin (27.3%), and 1 isolate to aztreonam, nalidixic acid and colistin (9.1% each).