Food Microbiology 82 (2019) 107–110

Contents lists available at ScienceDirect

Food Microbiology

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

Oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* associated with processed food in Europe

Narciso M. Quijada[a,b], Marta Hernández[a,b], Elena-Alexandra Oniciuc[c], José María Eiros[d], Isabel Fernández-Natal[e,f], Martin Wagner[g,h], David Rodríguez-Lázaro[b,*]

[a] Laboratorio de Biología Molecular y Microbiología, Instituto Tecnológico Agrario de Castilla y León (ITACyL), Valladolid, Spain
[b] Department of Biotechnology and Food Science, Faculty of Sciences, University of Burgos, Burgos, Spain
[c] Faculty of Food Science and Engineering, Dunarea de Jos University of Galati, Romania
[d] Department of Clinical Microbiology, Hospital Universitario Río Hortega, Valladolid, Spain
[e] Complejo Asistencial Universitario de León, León, Spain
[f] Institute of Biomedicine (IBIOMED), University of León, León, Spain
[g] Institute of Milk Hygiene, Milk Technology and Food Science, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria
[h] Christian Doppler Laboratory for Molecular Food Analytics, University of Veterinary Medicine, Vienna, Austria

ARTICLE INFO

Keywords:
Methicillin resistant *Staphylococcus aureus*
MRSA
Oxacillin-susceptible *mecA*-positive *Staphylococcus aureus*
OS-MRSA
Food

ABSTRACT

We report for the first time an oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* (OS-MRSA) associated with a processed food product in Europe. One isolate (MRSA-ST5-type V SCC*mec*) was found in cheese among 600 food samples confiscated from air passengers from international flights in Vienna Airport (Austria). Type V SCC*mec* strains do not harbor functional *mecI-mecR1* genes and in such strains *mecA* expression is regulated by the *bla* system (*blaI-blaR1-blaZ*). It has been recently reported that malfunctions in the *bla* system lead to the constitutive expression of *mecA*. The OS-MRSA reported in this study harbored the *bla* system on a plasmid and one deletion occurred in the *blaR1* gene causing a frameshift variant that lead to an incomplete BlaR1 protein. This finding highlights the potential role of food as a neglected route of dissemination of emerging MRSA variants.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an opportunistic pathogen associated with nosocomial and community acquired infections and recently being found in food producing animals and associated foodstuff (Oniciuc et al., 2015; Rodríguez-lázaro et al., 2015). MRSA is mediated by the expression of *mecA*, *mecB* or *mecC* genes, encoding a modified penicillin-binding protein (PBP) designated as PBP2a with reduced affinity for β-lactams or phenotypically having a minimum inhibitory concentration (MIC) of oxacillin higher than 4 μg/mL (CLSI, 2012; Conceição et al., 2015). However, some studies reported oxacillin-susceptible *S. aureus* strains classified by conventional phenotypic laboratory testing, but genotypically carrying the *mecA* gene (Chen et al., 2012; Pu et al., 2014; Conceição et al., 2015; Raji et al., 2016; Guimarães et al., 2017). Such strains have been defined as oxacillin-susceptible *mecA*-positive *S. aureus* (OS-MRSA) and due to misinterpretation within phenotypic studies, they can easily be misdiagnosed, potentially triggering the development of highly new resistant MRSA variants under antibiotic selection, due to the possession of *mecA* (Oniciuc et al., 2017).

The aim of this paper is to report, for the first time, the presence and the genetic background of an OS-MRSA associated with processed foods in Europe.

2. Identification of an oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* on processed foods

From August 2012 to March 2013, 600 foods confiscated at the International Vienna Airport (Austria) from passengers of international flights from non-EU countries were analyzed for the presence of MRSA (Rodríguez-Lázaro et al., 2017). The MIC for oxacillin of one *mecA* positive *S. aureus* strain (isolate LBMM3245) isolated from cheese carried by a passenger travelling from Cairo (Egypt) was in the susceptibility range by Microscan (Beckman Coulter S.L.U). This observation

* Corresponding author. Division of Microbiology, Department of Biotechnology and Food Science, Faculty of Sciences, University of Burgos, Plaza Misael Bañuelos s/n, 09001, Burgos, Spain.
E-mail address: drlazaro@ubu.es (D. Rodríguez-Lázaro).

https://doi.org/10.1016/j.fm.2019.01.021
Received 20 November 2018; Received in revised form 28 January 2019; Accepted 28 January 2019
Available online 30 January 2019
0740-0020/

Table 1
Phenotypic characterization of the OS-MRSA strain LBMM3245.

Test	Result
Coagulase production	Positive
MIC for erythromycin (μg/mL)	> 4
MIC for penicillin (μg/mL)	> 0.25
MIC for oxacillin (μg/mL)	0.5
MIC for tetracycline (μg/mL)	> 8
Cefoxitin screening:	
- Disc diffusion (mm)	Sensitive (24.5)
- Brilliance MRSA 2 Agar	Sensitive

was further confirmed by both Vitek II automated system (Biomerieux, France) and Sensititre Gram Positive All-in-One Plate system (Thermo Scientific, UK). Further screening showed that LBMM3245 was susceptible to cefoxitin (30 μg/disk, Oxoid) by the disk diffusion method. The antibiotic susceptibility pattern also showed that the isolate was positive for inducible clindamycin resistance and tetracycline, resistant to penicillin, and exhibiting intermediary resistance to erythromycin. The phenotypic characteristics of the LBMM3245 are detailed in Table 1.

The OS-MRSA LBMM3245 genome was sequenced on a MiSeq (Illumina) as previously described (Hernández et al., 2017). Reads were quality filtered using Prinseq v.0.20.4 (Schmieder and Edwards, 2011) and assembled by using SPAdes v3.10 (Bankevich et al., 2012), resulting in a 2.83 Mbp draft genome (40 × depth) formed by 176 contigs (N_{50} = 47,882) and a GC content of 32.7%. The draft genome was annotated using RAST (Aziz et al., 2008), showing 2750 coding sequences and 63 RNAs. Genome analysis of LBMM3245 by using mlst v2.10 (T. Seemann, https://github.com/tseemann/mlst), SCCmecFinder v.1.2 (International Working Group on The Classification of Staphylococcal Cassette Chromosome Elements, 2009) and spaTyper v1.0 (Bartels et al., 2014), revealed ST 5, SCC*mec* V (5C2) and *spa*-type t688. The antibiotic resistance profile of LBMM3245 was predicted by screening the draft genome against the ResFinder (Zankari et al., 2012) database by using BLASTN (Zhang et al., 2000). Results are summarized in Table 2. The presence of virulence genes was inferred by BLASTN searches against the Virulence Factors DataBase (VFDB) (Chen et al., 2005), revealing the presence of several enterotoxin and hemolysin related genes, such as *sed* (the precursor of staphylococcal enterotoxin D which is responsible for the symptoms of food poisoning) *hly/hla*, *hlgABC* and *hld* (α-, γ- and δ-hemolysin genes, respectively). γ-hemolysin has a role similar to that of Panton Valentine Leukocidin (PVL). PVL genes *lukE* and *lukF* were not detected, which is in concordance with other environmental OS-MRSA isolates published so far.

3. Analysis of the mechanisms involved in methicillin resistance in *Staphylococcus aureus* strain LBMM3245

The *mecA* gene in LBMM3245 showed 99.95% identity with *mecA* from ResFinder (GenBank accession number AB512767) and BLASTN search of the contig containing *mecA* against the GenBank database showed highest similarity to SCC*mec* type V *S. aureus* TSGH17 (GenBank accession number AB512767) (Hisata et al., 2011). Variant calling performed against *S. aureus* TSGH17 by using Bowtie2 v.2.3.2 (Langmead and Salzberg, 2012), samtools v.1.4.1 (Li et al., 2009) and VarScan v.2.3.9 (Koboldt et al., 2012) showed one single-nucleotide polymorphism (SNP) in position 1771 of *mecA* gene (T → C), that leads to an amino acid change from serine to proline at position 591 (S591P) in PBP2a, which resides in the transpeptidase (non-penicillin-binding) domain. BLAST searches in the GenBank database showed that the PBP2a nucleotide and amino acid sequences from LBMM3245 were unique in the GenBank database.

Table 2
Identification of antimicrobial resistance genes using ResFinder database.

Gene	Coverage (%)	Identity (%)	AN[a]	Predicted phenotype
blaZ	100	99.05	AJ302698	β-lactam resistance
erm(C)	100	100	M13761	Macrolide resistance
fexA	100	99.72	AJ549214	Phenicol resistance
fosD	100	79.05	KC989517	Fosfomycin resistance
mecA	100	99.95	AB512767	β-lactam resistance
tet(K)	100	99.93	U38428	Tetracycline resistance
tet(M)	100	99.58	X92947	Tetracycline resistance

[a] GenBank accession number.

SCC*mec* type V possesses the class C *mec* gene complex, which does not contain a *mecI* gene, and its *mecR1* gene is truncated and non-functional. In the absence of functional *mecI-mecR1* genes, *mecA* gene expression is regulated by the *blaI-blaR1* system (Sabat et al., 2015). *blaI, blaR1* and *blaZ* were found in a 27,395 bp contig in LBMM3245. PlasmidFinder v.1.3 (Carattoli et al., 2014) analysis revealed the presence of *rep*A (SAP074A) (*rep*-family 20; GenBank accession number GQ900426) in the same contig of the *bla* system. A GenBank search using BLASTN showed that the nucleotide sequence of the contig containing the *bla* system was most similar (> 99.8% nucleotide identity) to the 27,268 bp length plasmid pSAP074A (GenBank accession number GQ900426). (Fig. 1). PLACNETw (Vielva et al., 2017) was used to investigate the plasmid content in LBMM3245, revealing the presence of a chromosome and two putative plasmids, supported by the presence of *rep20-repA* (SAP074A) and *rep10-CDS1*(pIM13) (GenBank accession number M13761, respectively, as identified by using PlasmidFinder. (Fig. 2).

Variant calling of the contig containing the *bla* system in LBMM3245 using pSAP074A as reference revealed 10 variants (7 SNPs, 1 insertion, 2 deletions). One deletion occurred in nucleotide position 466 of *blaR1* causing a frameshift variant that leads to amino acid changes N158I, I159L and finally a stop codon in amino acid position 160, leading to an incomplete BlaR1 protein.

4. Discussion and conclusions

The appearance of oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* (OS-MRSA) has been reported worldwide in clinical isolates (Chen et al., 2012; Conceição et al., 2015) and in animal-associated foodstuffs (Pu et al., 2014; Raji et al., 2016; Guimarães et al., 2017). OS-MRSA strains can be misidentified by routine clinical laboratories, potentially triggering the development of new resistant MRSA variants and β-lactam treatment failure (Pournaras et al., 2013). To our knowledge, this is the first report on the presence of OS-MRSA on processed foods in Europe. This finding together with previous results obtained in our group (Oniciuc et al., 2015; Rodríguez-lázaro et al., 2015, 2017) draws attention to a neglected dissemination route of MRSA via the entrance of illegal food in Europe.

The OS-MRSA isolate LBMM3245 was found in a cheese confiscated from an air passenger travelling from Cairo, Egypt. It has an oxacillin MIC in the susceptible range of the EUCAST breakpoint (< 2 μg/mL), indicating that presence of *mecA* gene does not confer a high level resistance to oxacillin. Based only on the result of conventional antimicrobial susceptibility tests such as Microscan, VITEK, or Sensitrite screening, the isolate could have been misinterpreted as methicillin-sensitive *S. aureus* (MSSA).

Methicillin-resistance in MRSA is conferred by the expression of *mecA*, *mecB* or *mecC* genes (regulated by *mecR1-mecI*), encoding a modified penicillin-binding protein (designated as PBP2a) (Conceição et al., 2015). Genome analysis of the isolate LBMM3245 revealed a novel amino acid substitution (S591P) in the transpeptidase (non-penicillin-binding) domain of PBP2a. Amino acids variants in the non-penicillin-binding domain have been previously reported in other MRSA isolates and does not affect methicillin resistance (Mendes et al., 2012; Sabat et al., 2015).

In the absence of functional *mecI-mecR1* genes (as in type V

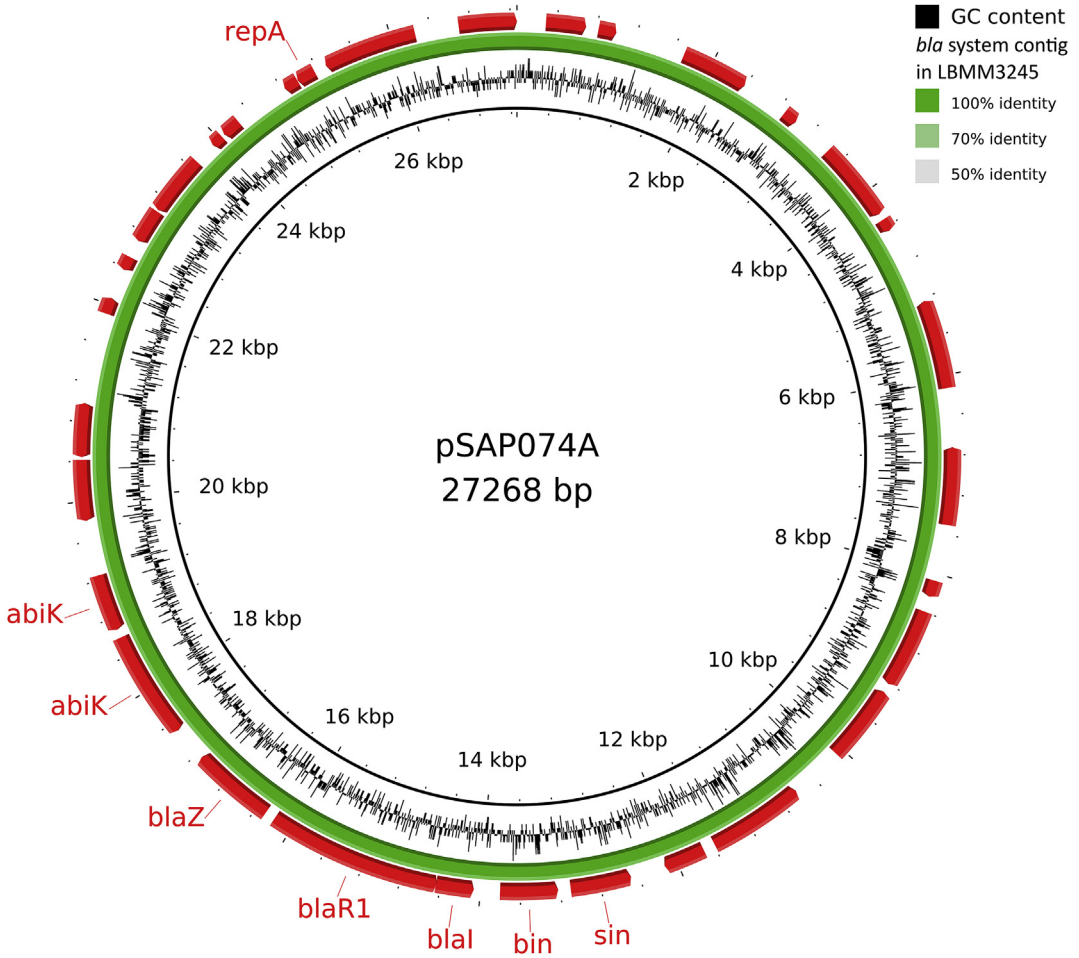

Fig. 1. Comparison of the 27,395 bp containing *bla* system in OS-MRSA strain LBMM3245 and plasmid pSAP074A (27,268 bp, GenBank accession number GQ900426). Image performed by using BRIG (Alikhan et al., 2011).

SCC*mec*), *mecA* expression is regulated by the *bla* system, which is usually carried on a plasmid or located on a transposon. BlaI can bind to the operator region of *mecA* and repress its transcription (Sabat et al., 2015). We found that the *bla* system together with *repA-rep20* in LBMM3245 present in a 27,395 bp contig was highly similar to plasmid pSAP074A. We also found a deletion in *blaR1* that led to a premature stop codon in BlaR1. In accordance with the experiments performed by Sabat et al. (2015), this incomplete BlaR1 might not be able to successfully cleave BlaI in the presence of β-lactams, leading to the constitutive repression of *mecA* and the oxacillin susceptibility of LBMM3245.

In conclusion, we report for the first time the presence of OS-MRSA

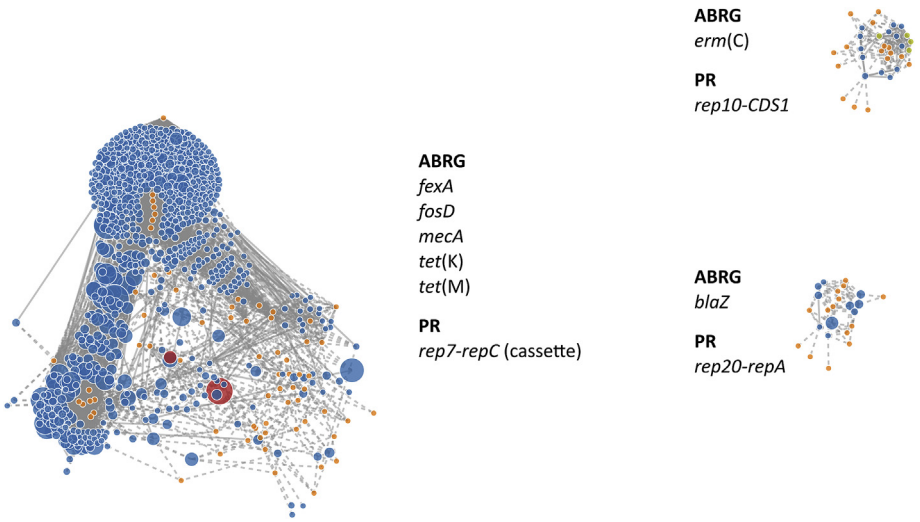

Fig. 2. PLACNETw investigation of the plasmid content of the OS-MRSA strain LBMM3245. Antibiotic resistance genes (ABRG) and plasmid replicons (PR) found in each constellation are shown.

in a processed food in Europe. Although this MRSA variant seems to be rare, it is of particular public health relevance because of its potential for the development of highly resistant MRSA under treatment with β-lactam antibiotics, and because it might not be detected by standard test procedures. The demonstration that new emerging MRSA variants, such as OS-MRSA, are already present in food in Europe, highlights that food should not be neglected as a route of MRSA transmission, as well as the need for monitoring the presence and evolution of OS-MRSA in food and environmental reservoirs.

Acknowledgements

This study was supported by the 7th EU Framework Programme PROMISE project (project n. 265877). DRL acknowledges the support by the Project AGL2016-74882-C3 of the Ministry of Economy and Competitiveness, Government of Spain. NMQ received a PhD fellowship from the Spanish National Institute for Agriculture and Food Research and Technology (INIA, Ministerio de Economía, Industria y Competitividad; fellowship FPI2014-020). This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession QBKY00000000. The version described in this paper is version QBKY00000000-01.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.01.021.

References

Alikhan, N.F., Petty, N.K., Ben Zakour, N.L., Beatson, S.A., 2011. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12, 402.

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., Formsma, K., Gerdes, S., Glass, E.M., Kubal, M., Meyer, F., Olsen, G.J., Olson, R., Osterman, A.L., Overbeek, R.A., McNeil, L.K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G.D., Reich, C., Stevens, R., Vassieva, O., Vonstein, V., Wilke, A., Zagnitko, O., 2008. The RAST server: rapid annotations using subsystems Technology. BMC Genomics 9, 75. https://doi.org/10.1186/1471-2164-9-75.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477. https://doi.org/10.1089/cmb.2012.0021.

Bartels, M.D., Petersen, A., Worning, P., Nielsen, J.B., Larner-Svensson, H., Johansen, H.K., Andersen, L.P., Jarløv, J.O., Boye, K., Larsen, A.R., Westh, H., 2014. Comparing whole-genome sequencing with Sanger sequencing for *spa* typing of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. 52 4305 LP-4308.

Carattoli, A., Zankari, E., García-Fernández, A., Larsen, M.V., Lund, O., Villa, L., Aarestrup, F.M., Hasman, H., 2014. In Silico detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing. Antimicrob. Agents Chemother. 58, 3895–3903. https://doi.org/10.1128/AAC.02412-14.

Chen, F., Huang, I., Wang, C., Chen, P., Wang, H., Lai, J., Shiau, Y., Lauderdale, T.Y., 2012. *mecA*-positive *Staphylococcus aureus* with low-level oxacillin MIC in Taiwan. J. Clin. Microbiol. 50 (5), 1679–1683.

Chen, L., Yang, J., Yu, J., Yao, Z., Sun, L., Shen, Y., Jin, Q., 2005. VFDB: a reference database for bacterial virulence factors. Nucleic Acids Res. 33 (Suppl. l_1), D325–D328.

CLSI, 2012. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard - Eleventh Edition. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard - Eleventh Edition. CLSI, Wayne, USA.

Conceição, T., Coelho, C., de Lencastre, H., Aires-de-Souza, M., 2015. Frequence occurence of oxacillin-susceptible mecA positive *Staphylococcus aureus* (OS-MRSA) strains in two African countries. J. Antimicrob. Chemother. 70 (12), 3200–3204.

Guimarães, F.F., Manzi, M.P., Joaquim, S.F., Richini-Pereira, V.B., Langoni, H., 2017. Outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA)-associated mastitis in a closed dairy herd. J. Dairy Sci. 100 (1), 726–730. https://doi.org/10.3168/jds.2016-11700.

Hernández, M., Iglesias, M.R., Rodríguez-Lázaro, D., Gallardo, A., Quijada, N.M., Miguela-Villoldo, P., Campos, M.J., Píriz, S., López-Orozco, G., de Frutos, C., Sáez, J.L., Ugarte-Ruíz, M., Domínguez, L., Quesada, A., 2017. Co-occurrence of colistin-resistance genes *mcr*-1 and *mcr*-3 among multidrug-resistant *Escherichia coli* isolated from cattle, Spain, September 2015. Euro Surveill. 22 (31), pii = 30586. https://doi.org/10.2807/1560-7917.ES.2017.22.31.30586.

Hisata, K., Ito, T., Matsunaga, N., Komatsu, M., Jin, J., Li, S., Watanabe, S., Shimizu, T., Hiramatsu, K., 2011. Dissemination of multiple MRSA clones among community-associated methicillin-resistant Staphylococcus aureus infections from Japanese children with impetigo. J. Infect Chemoter. 17, 609–621. https://doi.org/10.1007/s10156-011-0223-4.

International Working Group on The Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), 2009. Classification of staphylococcal cassette chromosome mec (SCC*mec*): guidelines for reporting novel SCCmec elements. Antimicrob. Agents Chemother. 56 (12), 4961–4967.

Koboldt, D.C., Zhang, Q., Larson, D.E., Shen, D., McLellan, M.D., Lin, L., Miller, C.A., Mardis, E.R., Ding, L., Wilson, R.K., 2012. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res. 22, 568–576. https://doi.org/10.1101/gr.129684.111.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie2. Nat. Methods 9 (4), 357–359. https://doi.org/10.1038/nmeth.1923.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078–2079.

Mendes, R.E., Tsakris, A., Sader, H.S., Jones, R.N., Biek, D., McGhee, P., Appelbaum, P.C., Kosowska-Shick, K., 2012. Characterization of methicillin-resistant *Staphylococcus aureus* displaying increased MICs of ceftaroline. J. Antimicrob. Chemother. 67, 1321–1324.

Oniciuc, E., Ariza-miguel, J., Bolocan, A., Diez-valcarce, M., Rovira, J., Hernández, M., Fernández-natal, I., Ioana, A., Rodríguez-lázaro, D., 2015. Foods from black market at EU border as a neglected route of potential methicillin-resistant *Staphylococcus aureus* transmission. Int. J. Food Microbiol. 209, 34–38. https://doi.org/10.1016/j.ijfoodmicro.2014.11.015.

Oniciuc, E.A., Nicolau, A.I., Hernández, M., Rodríguez-Lázaro, D., 2017. Presence of methicillin-resistant *Staphylococcus aureus* in the food chain. Trends Food Sci. Technol. 61, 49–59. https://doi.org/10.1016/j.tifs.2016.12.002.

Pournaras, S., Stathopoulos, C., Tsakris, A., 2013. Oxacillin-susceptible MRSA: could it become a successful MRSA type? Future Microbiol. 8 (11), 1365–1367.

Pu, W., Su, Y., Li, J., Li, C., Yang, Z., Deng, H., Ni, C., 2014. High incidence of oxacillin-susceptible mecA-positive *Staphylococcus aureus* (OS-MRSA) associated with bovine mastitis in China. PLoS One 9 (2), e88134. https://doi.org/10.1371/journal.pone.0088134.

Raji, M.A., Garaween, G., Ehricht, R., Monecke, S., Shibl, A.M., Senok, A., 2016. Genetic characterization of *Staphylococcus aureus* isolated from retail meat in riyadh, Saudi Arabia. Front. Microbiol. 7, 911.

Rodríguez-lázaro, D., Ariza-miguel, J., Diez-valcarce, M., Fernández-natal, I., Hernández, M., Rovira, J., 2015. Foods confiscated from non-EU flights as a neglected route of potential methicillin-resistant *Staphylococcus aureus* transmission. Int. J. Food Microbiol. 209, 29–33. https://doi.org/10.1016/j.ijfoodmicro.2014.08.016.

Rodríguez-Lázaro, D., Oniciuc, E.-A., García, P.G., Gallego, D., Fernández-Natal, I., Dominguez-Gil, M., Eiros-Bouza, J.M., Wagner, M., Nicolau, A.I., Hernández, M., 2017. Detection and characterization of Staphylococcus aureus and methicillin-resistant S. aureus in foods confiscated in EU borders. Front. Microbiol. 8, 1344. https://doi.org/10.3389/fmicb.2017.01344.

Sabat, A.J., Pournaras, S., Akkerboom, V., Tsakris, A., Grundmann, H., Friedrich, A.W., 2015. Whole-genome analysis of an oxacillin-susceptible CC80 mecA-positive *Staphylococcus aureus* clinical isolate: insights into the mechanisms of cryptic methicillin resistance. J. Antimicr. Chemoter. 70, 2956–2964.

Schmieder, R., Edwards, R., 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics 27 (6), 863–864.

Vielva, L., de Toro, M., Lanza, V.F., de la Cruz, F., 2017. PLACNETw: a web-based tool for plasmid reconstruction from bacterial genomes. Bioinformatics 33 (23), 3796–3798.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F.M., Larsen, M.V., 2012. Identification of acquired antimicrobial resistance genes. J. Antimicrob. Chemother. 67, 2640–2644. https://doi.org/10.1093/jac/dks261.

Zhang, Z., Schwartz, S., Wagner, L., Miller, W., 2000. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. 7 (1–2), 203–214. https://doi.org/10.1089/10665270050081478 PMID: 10890397.