Prevalence, Antimicrobial Susceptibility and Virulence Factors Profile of Methicillin- resistant *Staphylococcus aureus* (MRSA) on Food in Portugal

A. Castro, C. Palhau, S. Cunha, S. Camarinha, J. Silva, P. Teixeira*

CBQF – Centro de Biotecnologia e Química Fina, Escola Superior de Biotecnologia, Centro Regional do Porto da Universidade Católica Portuguesa, Rua Dr. António Bernardino Almeida, 4200-072 Porto, Portugal

*pcteixeira@porto.ucp.pt

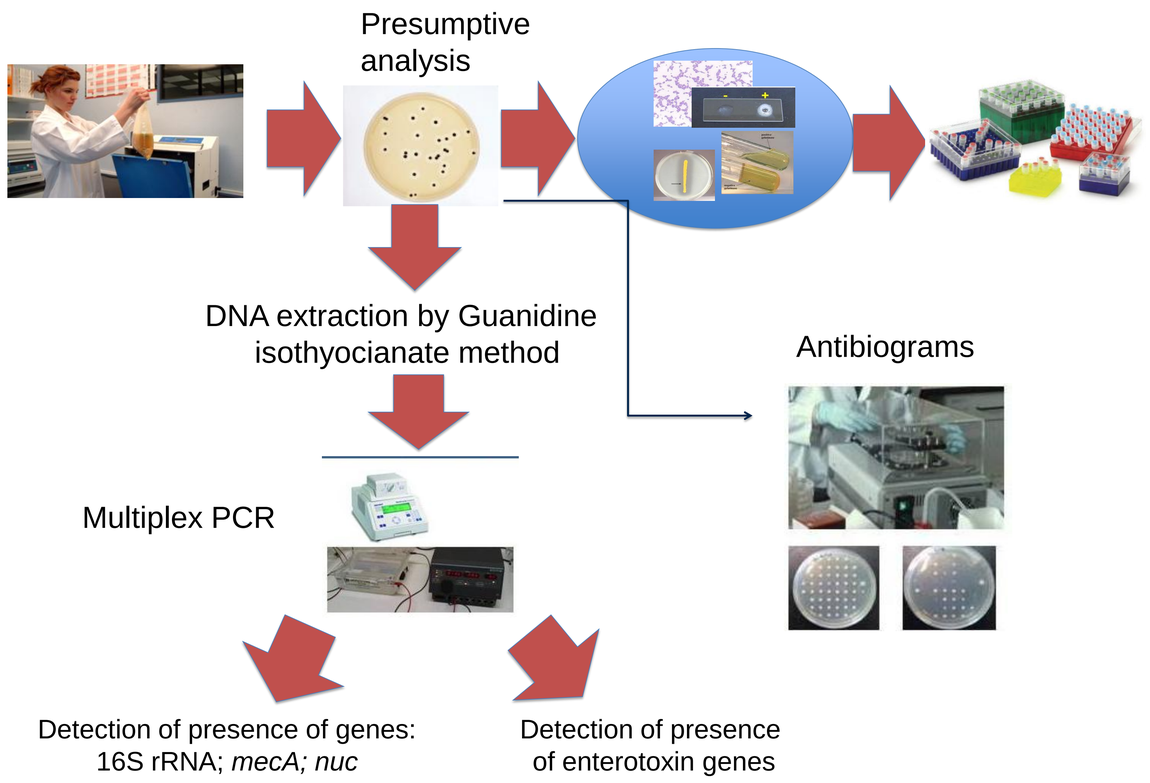
Objective

The purpose of this study was to evaluate the presence of *Staphylococcus aureus* in foods, to determine to what extent isolates are resistant to a range of antibiotics and the proportions of MRSA strains. An indication of the potential degree of virulence was obtained by determination of the presence of hemolysins, lipase, gelatinase and enterotoxins genes.

Introduction

Staphylococcus aureus is one of the most important pathogens responsible for foodborne intoxications [1,2]. The primary habitat of *S. aureus* is the mucous membranes of the human nasopharynx and animal skin [3]. The presence of *S. aureus* in foods is often related to improper handling by personnel, who are frequently contaminated with or carriers of these microorganisms. Eleven major antigenic types of SEs have been recognised (SEA to SEJ) and their corresponding genes have been reported [1]. Recently, other SE toxins were identified (SEK, SEL, SEM, SEN, SEO, and SEU) and the corresponding genes described, but their role in food poisoning is not clear [1]. In the last decades, the spread of antibiotic resistance in bacteria, including staphylococci, is increasing and may represent a hazard for human health. Among antibiotic resistant staphylococci, multidrug-resistant *S. aureus* strains are of great clinical and public concern since resistances make the treatment of infections much more difficult. The *mecA* gene is harboured on the staphylococcal chromosomal cassette *mec* (*SCCmec*), a genetic element that integrates site specifically into the *S. aureus* chromosome [4]. MRSA strains are also becoming more frequent in foods [2]. Food may then be considered an excellent way of introducing pathogenic microorganisms into the general population and into immuno-compromised people, and thereby transfer antibiotic-resistant bacteria to the intestinal tract of consumers, very efficiently [2]. It is in the intestine that the transfer of resistance genes can occur between non-pathogenic bacteria and pathogenic or opportunist bacteria [2].

Methods



Results and discussion

From 2008 to 2009, different food products, mainly from the North of Portugal were submitted to a routine microbiology laboratory. One hundred and sixteen presumptive *S. aureus* on Baird Parker agar with rabbit plasma fibrinogen were confirmed by Gram staining, presence of catalase and coagulase, growth on Mannitoll Salt Agar (MSA) and presence of DNAse. Confirmation and characterization of *S. aureus* was performed by PCR multiplex (figure 1) detecting simultaneous presence of presence of 16SrRNA (*Staphylococcus* genus specific), *nuc* (*S. aureus* species specific) and *mecA* (determinant of MET resistance) genes.

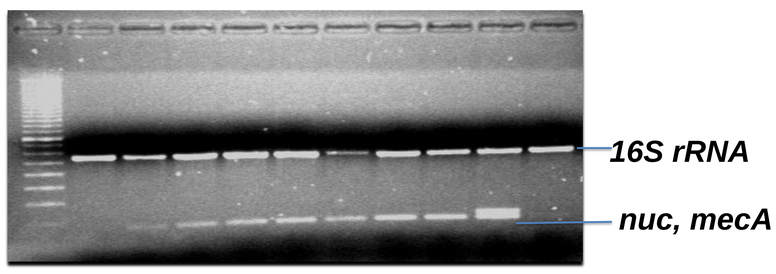


Figure 1: PCR detection of the presence of genes 16S rRNA; *mecA; nuc*

MRSA

38.8% of the isolates were resistant to oxacillin and therefore classified as MRSA. According to the detection of *mecA* gene in this study, only three isolates showed a positive result (2.6%). These isolates should have acquired resistance by *mecA* gene. The other isolates could have acquired resistance to oxacillin by other mechanisms.

Table I: Percentages **of hemolisins, lipase and gelatinase among MRSA** isolates

hemolisins			lipase +	gelatinase +
β	α	γ		
45.9%	16.2%	37.8%	59.5%	94.6%

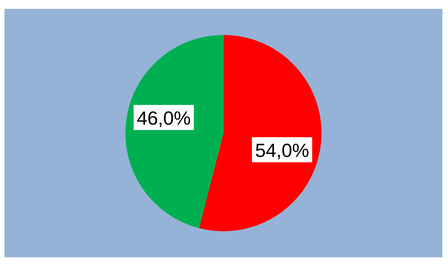


Figure 4: Percentage of enterotoxigenic and non enterotoxigenic MRSAs

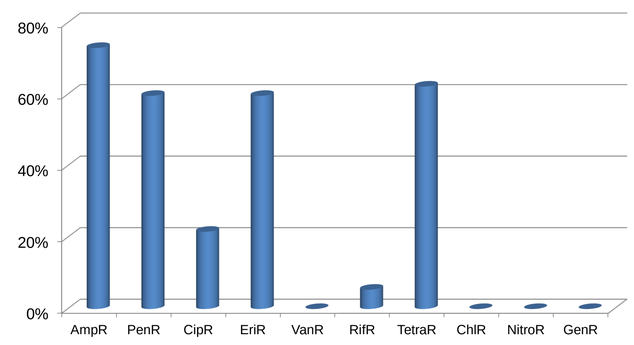
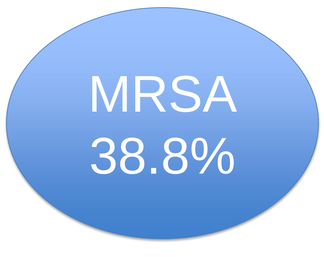


Figure 2: Percentage of resistant of MRSAs to ten antibiotics tested



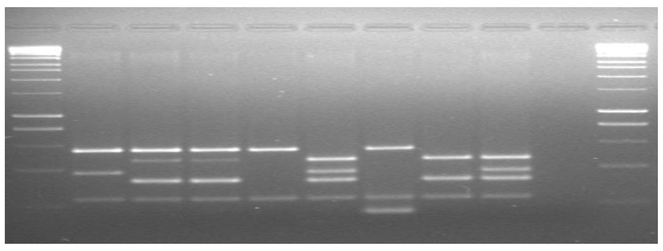


Figure 3: PCR detectiion of the presence of enterotoxin genes

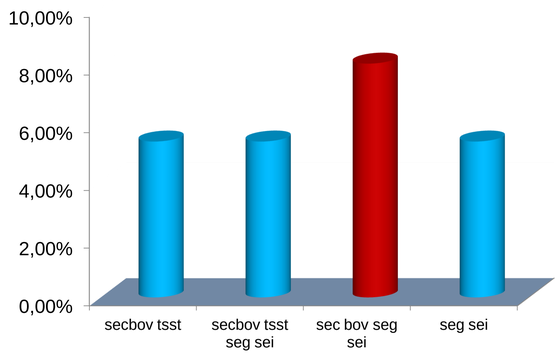


Figure 5: Most prevalent combinations of enterotoxin genes

Conclusions

Staphylococcus aureus is well established as a clinical and epidemiological pathogen; in this study it was demonstrated that the potentially pathogenic role of *S. aureus* as a food-borne pathogen should not be neglected. In conclusion, these results highlight the potentially high risk for consumers in the absence of strict hygienic and preventative measures to avoid the presence of *S. aureus* and SEs production in foods, emphasizing the need for improved hygiene practices during food processing and also during the distribution and consumption of the final food products.

References

[1] S. Morandi,M. Brasca,C. Andrighetto,A. Lombardi,R. Lodi, International Journal of Microbiology, 2009,1-7 2]G. Pesavento, B. Ducci, N. Comodo, A. Lo Nostro, Food Control, 2007, 18, 196-200. [3] J Kluytmans, W. van Leeuwen, W. Goessens, R. Hollis,S.Messer, L. Herwaldt, H. Bruining, M. Heck, J. Rost, N. van Leeuwen, A. van Belkum, and H. Verbrugh, Journal of Clinical Microbiology, 1995, 33, 5, 1121–1128 4] Boyle-Vavra, S. and Daum, R.S. Laboratory Investigation, 2007 87:3-9. [5] K. Zhang, J. Sparling, B. Chow, S. Elsayed, Z. Hussain, D. Church, D. Gregson, T. Louie, J.Conly, Journal of Clinical Microbiology, 2004, 42, 11, 4947–4955. 6] Aires de Sousa, M., Sanches, I.S., van Belkum, A., van Leeuwen, W., Verbrugh, H. and de Lencastre, H. Microbial Drug Resistance., 1996 2(3):331-341. [7] CLSI, Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement. CLSI document M100-S15. 2007, Clinical and Laboratory Standards Institute, Wayne, Pa. [8] V. Pereira, C. Lopes, A. Castro, J. Silva, P. Gibbs, P. Teixeira, Food Microbiology, 2009, 26, 278–282.

Governo da República Portuguesa

Acknowledge

This work was supported by National Funds from FCT – Fundação para a Ciência e a Tecnologia through project PEst-OE/EQB/LA0016/2011. FCT for PhD grant BD/39315/2007 to Ana Isabel Castro.