

Avaliação da prevalência da colonização por staphylococcus aureus em trabalhadores de uma padaria: Estudo de caso
Assessment of staphylococcus aureus colonization in bakery workers: A case study

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Resumo

A colonização humana por staphylococcus aureus é mundialmente generalizada entre a comunidade e considerada um factor de alto risco para o desenvolvimento de infecções clínicas em indivíduos saudáveis ou em indivíduos imunocomprometidos. Os humanos colonizados são assim um reservatório bacteriano com associado alto risco de transmissão de colonização e infecção para outros indivíduos, ou de contaminação de alimentos e superfícies alimentares durante o manuseio dos mesmos. A ingestão de enterotoxinas estafilocócicas presentes nos alimentos pode originar intoxicação alimentar estafilocócica (SFP) a qual está associada a sintomas graves, tais como vômitos severos, náuseas, diarreia e prostração. Considerando que a contaminação de alimentos por manipuladores de alimentos com colonização nasofaríngea representa um grande risco para SFP, neste trabalho pretendemos avaliar a prevalência da colonização de estirpes sensíveis e resistentes à meticilina de S. aureus numa padaria como estudo de caso. Relatamos uma prevalência elevada de S. aureus (40%) nos trabalhadores avaliados, dos quais 75% das estirpes identificadas foram sensíveis à meticilina (MSSA) e 25% resistentes (MRSA).

Este trabalho fomenta a conscientização para a impreterível necessidade de desenvolver programas de vigilância em saúde ocupacional com objectivo de prevenir a contaminação de alimentos no contexto da Saúde Pública.

Palavras-chave: Colonização nasofaríngea; trabalhadores de padaria; staphylococcus aureus; resistência à meticilina.

Abstract

Human colonization by staphylococcus aureus is widespread among the community and is considered a high-risk factor for the development of clinical infections in either healthy or immunocompromised individuals. Human carriers become a bacterial reservoir with associated high risk to transfer the infection to others, or contaminate foods and food surfaces during handling. The ingestion of staphylococcal enterotoxins present in food can lead to staphylococcal food poisoning (SFP) which can cause severe symptoms such as violent emesis, nausea, diarrhea and prostration. Considering that food contamination by colonized food handlers represent a major risk for SFP, here we aim to assess S. aureus colonization prevalence of both sensible and resistant strains in a bakery as a case study. We report elevated prevalence of

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S. aureus prevalence (40%) in bakery workers whereas 75% of identified strains were sensible to methicillin (MSSA) and 25% resistant (MRSA).

This work raises awareness to the urge of creating valuable occupational health surveillance programs to avoid food contamination in the context of Public Health.

Keywords: Nasopharyngeal colonization; food handlers; staphylococcus aureus; methicillin resistance.

1. Introduction

The worldwide increase in antibiotic resistant microorganisms reported in the past decade (Morris and Masterton, 2002), sustain emergent social and scientific concerns regarding the continuous over prescription and extensive use of antibiotics (Smith *et al.*, 2002). Currently, the World Health Organization (WHO) describes antimicrobial resistance in human pathogens as a global health challenge (WHO, 2016). Besides clinical infections, resistant microorganisms can also be found in healthy individuals that become carriers of these potentially pathogenic agents without necessarily presenting characteristic signs and symptoms of infection (asymptomatic carrier). Human colonization is considered a high risk factor for the development of infections, particularly in the case of staphylococcus aureus colonization (Ghasemzadeh-Moghaddam *et al.*, 2015). Nasopharyngeal microbiota represents the primary site of colonization for this pathogenic agent. *S. aureus* is a gram-positive, spherical and pyogenic bacteria with extremely high capacity to rapidly disseminate in diverse environmental contexts, transmitted by either direct (particularly hands) or indirect (contaminated surfaces or fomites) contact (Davis *et al.*, 2012). These microorganisms are characterized by their ability to infect both healthy or immunocompromised individuals with associated exponentially increasing morbi-mortality rates (Tong *et al.*, 2015), severe prognosis in invasive blood-stream and pneumonic infections (Klevens *et al.*, 2007), frequently allied to antibiotic resistance, specifically methicillin resistance (methicillin-resistant *S. aureus* - MRSA). Human MRSA carriers have increased risk for subsequent occurrence of clinical disease (up to 10-fold) and become a bacterial reservoir with associated high risk to transfer the infection to others, or contaminate foods and food surfaces during handling (Jordan *et al.*, 2011). Besides nosocomial infections, *S. aureus* is also a cause of staphylococcal food poisoning (SFP), resultant from ingestion of staphylococcal enterotoxins present in food, with associated symptoms such as violent emesis, nausea, diarrhea and prostration. SFP is one of the most prevalent foodborne intoxications in the world, with fatality rates ranging from 0.03% in the general population to 4.4% in children and the elderly (Wattinger *et al.*, 2012).

Considering that foodstuff contamination by colonized food handlers represent a major risk for SFP and a recently published study have reported high colonization and hand contamination rates of *S. aureus* in food handlers workers (Ho *et al.*, 2015), here we aim to evaluate the prevalence of *S. aureus* colonization of both methicillin sensible and resistant strains, in workers of a bakery as a case study.

2. Materials and Methods

Study population

The study included workers from a bakery analyzed for occupational-associated colonization of *S. aureus* sensible and resistant to methicillin. The study sample totaled 10 nasopharyngeal swabs from workers that provided a signed written informed consent before enrolment in the study. The individuals enrolled in the study also answered to a questioner for collection of demographic data, daily occupational activity and duration (years), availableness of individual protection equipment at the workplace and daily regular and/or persistent cough episodes.

Collection, isolation and microbiological procedures

Biological samples were obtained through nasopharyngeal swab procedure using transport swabs with Stuart media. The introduction of the swab for biological harvest was done along the septum to 2.5 cm from the external orifice (up to "feel " a slight resistance) and rotated several times before removing it and immediately transported to the laboratory at room temperature. In the microbiology laboratory samples were cultured in flat petri dishes containing Columbia agar with 5% sheep blood, and incubated for 24 hours at 37°C. After the incubation period, colonies were evaluated based on cultural characteristics and suspicious colonies were isolated the same culture media and incubated for 24 hours at 37° C. Identification of *S. aureus* was performed in isolated colonies trough catalase test and Slidex Staph Kit (REF). MRSA strains were identified through isolation of the strains in chromID® MRSA agar (Sigma ref# 90923) and confirmed by Slidex MRSA detection Test Kit (Biomerieux ref #73117). In this work positive (*S. aureus* MRSA laboratory collection) and negative (*S. aureus* ATCC 25923) control strains will be included as positive and negative controls. *Streptococcus pneumoniae* suspicious colonies were also isolated and identification was performed trough Slidex *S. pneumoniae* detection Test Kit (Biomerieux ref #58811).

Ethical aspects

The study performance respected all ethical precepts that rule human research.

3. Results

Bakery workers encompass a high prevalence of *S.aureus* colonization

Bakery workers enrolled in the study (n=10) comprised 2 women and 8 caucasian men aged between 32 and 55 years old. Individuals perform occupational activity during an average of 8 daily hours during 3 to 40 years. Although all the workers stated that individual protection equipment is available in the work place, none of them utilize personal protection masks. Regarding regular and persistent cough occurrences only one individual has reported chronic episodes.

In all studied individuals normal commensal flora, namely *Staphylococcus* spp. and *Streptococcus* spp. was observed. The prevalence of *S. aureus* in the studied bakery was 40%. Regarding the resistance profile, 75% of *S. aureus* strains were sensible to methicillin (MSSA) and 25% resistant (MRSA) as

described in table 1. It is important to notice that the worker that reported regular and persistent cough occurrences is a MSSA carrier. Furthermore, other potentially pathogenic bacteria namely gram positive *Streptococcus pneumoniae* (10%) and gram negative *Proteus spp.* (10%) were also identified.

Table 1- Identified pathogenic bacteria

Bacteria	Prevalence	Percentage
<i>Staphylococcus aureus</i>	4/10	40%
<i>Staphylococcus aureus</i> MSSA	3/4	75%
<i>Staphylococcus aureus</i> MRSA	1/4	25%
<i>Streptococcus pneumoniae</i>	1/10	10%
<i>Proteus spp.</i>	1/10	10%

4. Discussion

Community-acquired *S. aureus* colonization is spread worldwide including methicillin-resistant strains. Bacteria as well as microbial products such as endotoxins are key components of bioaerosols which may occur as solid or liquid particles in the air. Prolonged exposure to bioaerosols particularly at workplaces can represent a health hazard and potentially result in infectious disease (Walser *et al.*, 2015) either for workers and for the spread of these microorganisms in the community. For the past years, numerous studies have increased scientific data on occupational exposure to bioaerosols and helped to understand the relationship between exposure and health effects (Ghosh Lal and Srivastava, 2015). In the context of food handlers, although human colonization with bacteria such as *S. aureus* is associated high risk to transfer the infection to others, or contaminate foods and food surfaces during handling (Jordan *et al.*, 2011), information regarding bacteria bioburden is still scarce. Considering that *S. aureus* is a cause of staphylococcal food poisoning (SFP), resultant from ingestion of staphylococcal enterotoxins present in food, is one of the most prevalent foodborne intoxications in the world, with fatality rates particularly concerning for children and the elderly (Wattinger *et al.*, 2012) and that foodstuff contamination by colonized food handlers represent a major risk for SFP (Ho *et al.*, 2015), the assessment of colonization of these workers is crucial.

In the presented case study, we reported a 40% prevalence of *S. aureus* asymptomatic carriers in food handlers, which is higher than levels detected in the community (31%) (Hatcher *et al.*, 2016). Additionally, our data revealed elevated prevalence of MRSA (10% in bakery workers in comparison to 2% - 3% in the community) (Hatcher *et al.*, 2016) which is particularly concerning considering that community-associated methicillin resistant strains were also associated with toxic shock syndrome cases (Sada *et al.*, 2017). Relevantly, the fact that one of the asymptomatic carriers of MSSA was the worker who assumed to have regular and persistent cough occurrences, the probability of spread of this microorganism through bioaerosols is higher.

Moreover, our results also evidence high prevalence levels of *S. aureus* in the studied workers in relation to the range reported recently by Ho *et al.* coworkers in 2003 before implementation of rigorous hygiene measures. In the referred study, authors described that *S. aureus* nasal carriage rate in Hong Kong in 2003 (35%), significantly decreased (23,5%) after strictly hygiene measures were implemented and prevalence were maintained until 2011 (22.9%). (Ho *et al.*

al., 2015). This study clearly demonstrated that reduction spread opportunities associated with improved hygiene measures is crucial for control of disease. In our case study, it is also important to notice that, the identification of *Proteus* spp., a Gram-negative bacteria found in human intestine and feces, commonly responsible for urinary and septic infections, often nosocomial, may indicate poor hygiene although colonization has been found in pig-exposed individuals (Fischer *et al.*, 2016).

Moreover, we have also identified normal commensal flora of upper respiratory tract, namely *Staphylococcus* spp. and *Streptococcus* spp., and *Streptococcus pneumoniae* in levels congruent with colonization reported in other studies (Aljicevic *et al.*, 2015).

Overall, the presented case study clearly demonstrates the urge to develop proper and valuable bioburden risk assessments, particularly in the context of food handlers, which can endorse major implications in public health.

5. Conclusion

The assessment of *Staphylococcus aureus* colonization in food handler personnel such as bakery workers is of foremost importance to evaluate potential sources of food contamination by these microorganisms. This work raises awareness to the necessity of creating valuable occupational health surveillance programs and proper procedures to avoid the occurrence of staphylococcal food poisoning (SFP).

6. References

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