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Analysis of home dust for *Staphylococcus aureus* and staphylococcal enterotoxin genes using quantitative PCR

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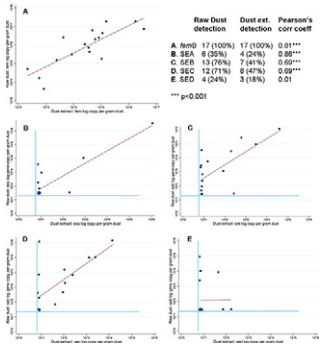
HIGHLIGHTS

- A method for evaluation of *S. aureus* and SEs in home dust is presented.
- DNA-based testing was more sensitive than culture methods.
- Staphylococcal exposures may be common in homes of urban adults with asthma.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: The bacterium *Staphylococcus aureus* (SA) is known to induce allergic inflammatory responses, including through secreted staphylococcal enterotoxin (SE) superantigens. To quantify indoor environmental exposures to these potential allergens, which may be associated with worse asthma, we developed a method for the assessment of *S. aureus* and SE in home dust and applied it to a study of homes of inner-city adults with asthma.

Methods: We conducted laboratory experiments to optimize sample processing and real-time PCR methods for detection and quantification of SA (*femB*) and SEA-D, based on published primers. We applied this method to dust and dust extract from 24 homes. We compared results from real-time PCR to culture-based results from the same homes.

Results: The bacteremia DNA isolation method provided higher DNA yield than alternative kits. Culture-based results from homes demonstrated 12 of 24 (50%) bedrooms were contaminated with *S. aureus*, only one of which carried a SE gene (SEC). In contrast, *femB* was detected in 23 of 24 (96%) bedrooms with a median of 1.1×10^6 gene copies detected per gram of raw dust. Prevalence and median copy number (shown in parenthesis) of SE gene detection in bedroom dust was: SEA 25% (1.4×10^2); SEB 63% (1.4×10^3); SEC 63% (1.1×10^3); SED 21% (1.3×10^2).

Conclusions: Our culture-independent method to detect *S. aureus* and SE in home dust was more sensitive than our culture-based method. Prevalence of household exposure to *S. aureus* and SE allergens may be high among adults with asthma.

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1. Introduction

Indoor home environmental exposures to biotic material, particularly allergens and microbial endotoxin, are known to exacerbate allergic asthma among people with existing disease (Matsui et al., 2008). The bacterium *Staphylococcus aureus* (SA) also may induce allergic inflammatory host responses, including through secreted staphylococcal enterotoxin (SE) superantigens (Bachert and Zhang, 2012). SA is known to aggravate eczema and a growing body of evidence suggests SA exposure may contribute to the development or exacerbation of a related disease, allergic asthma (Davis et al., 2015; Sintobin et al., 2015; Song et al., 2014; Bachert et al., 2012a). While microbial exposures have been linked to both protection against and risk for asthma development, it is not known whether SA and SE exposures within the home environment impact respiratory symptoms and lung function in people with asthma.

While methods are established to quantify home environmental allergen exposure, corresponding methods for SA/SE detection and quantification have not yet been validated. In prior studies, microbial DNA-based methods using quantitative PCR (qPCR) methods have demonstrated good reliability and applicability to large cohorts for assessment of microbial exposures in home dust samples (Fujimura et al., 2012; Kaarakainen et al., 2009; Rintala et al., 2008; Scherer et al., 2014). DNA-based methods may provide a less biased estimate of microbial exposures than culture-based methods, which require organism viability and cultivability (Kaarakainen et al., 2009). This may be particularly important for studies, not of acute infection, but of immune modulation in the context of chronic disease outcomes.

While qPCR methods exist to confirm cultured isolates as *S. aureus* and evaluate whether such isolates carry certain staphylococcal enterotoxin genes (Klotz et al., 2003), no prior study has evaluated these methods for use in the complex environmental matrix of home dust. Hence, we adapted and optimized a microbial DNA-based method for home dust assessment for SA/SE and applied this method, comparing results from qPCR assessment to culture-based results, in a pilot study of the homes of inner-city adults with asthma. We hypothesized that detection of SA would be more common than detection of SEs, which are variably carried by staphylococcal strains, and that gene-based detection would be more sensitive than culture-based detection methods.

2. Methods

2.1. Method evaluation and optimization (laboratory study)

2.1.1. Genomic DNA isolation

Two bead-beating DNA Purification kits were compared for DNA isolation performance: BiOstic® Bacteremia and Powerlyzer® Powersoil® (MoBio Laboratories, Carlsbad, CA, USA). Each kit was tested using three replicates of: 1) overnight-grown staphylococcal culture (*S. aureus* ATCC 13565) only; 2) 50 mg of standard house dust (National Institute of Standards and Technology, Gaithersburg, MD, USA) combined with overnight-grown staphylococcal culture ("spiked" dust); and 3) 50 mg of standard house dust only. A previous trial in our laboratory showed that qPCR fluorescence threshold values for DNA extracts from 50 mg aliquots of dust did not differ significantly from those from 100 mg aliquots. For each sample that contained staphylococcal culture, two different volumes of culture were tested using calibrated 1 μl and 10 μl inoculating loops (Fisher Scientific).

Cells from microbial culture were suspended in the bead solutions for each kit, and these suspensions were added to the bead tubes. When dust was used, it was added directly to the bead tube before adding staphylococcal cells from microbial culture. DNA isolation then proceeded according to the manufacturer instructions for each kit. Purified extracts were analyzed for DNA concentration using a Qubit™ 3.0 Fluorometer (LifeTechnologies).

2.1.2. Real-time PCR protocol

TaqMan PCR assays were run as duplex reactions to test for both the *femB* gene and a single SE gene in each reaction; therefore, four reactions were carried out for each sample in order to test for the presence of four different SE genes (A, B, C and D). The primers and TaqMan probes (Klotz et al., 2003) were synthesized by Eurofins Genomics (Huntsville, AL, USA), as previously published, except the *femB* probe was labeled with an alternate fluorophore, JOE, rather than FAM, to accommodate the duplex reaction. Reaction mixtures (25 μl final volume) contained 1× TaqMan Universal Master Mix II (no UNG) (Applied Biosystems™); 50 pmol each primer; 150 nM TaqMan probe; and 5–9 μl template DNA. Amplification was carried out using a StepOnePlus Real-Time PCR system (Applied Biosystems™) under the following parameters: 50 °C for 2 min; 95 °C for 10 min; 45 cycles of 95 °C for 15 s and 60 °C for 1 min.

2.1.2.1. Detection of femB and SEA genes in spiked dust samples. Real-time PCR was performed using 9 μl of DNA template isolated from the dust samples, as described above, to detect *fem*B and SEA genes present in the *S. aureus* ATCC 13565 strain used for the spiked dust experiment.

2.1.3. Quantification of femB/SE genes

Standard curves for gene copy number were generated for *femB* and each of SEA-D genes. Plasmids containing the target gene sequences were cloned using the Qiagen PCR CloningPlus Kit (Qiagen; used for enterotoxin genes) or the TOPO® TA Cloning Kit for Sequencing (Invitrogen; used for *fem*B), following manufacturer instructions. Plasmids were isolated from transformed cells using the PureLink Quick Plasmid Miniprep Kit (Invitrogen), following manufacturer instructions. Purified extracts were analyzed for DNA concentration as described above. Gene copy number per microliter of stock plasmid solution was then calculated. The stock solution was then serially diluted to obtain concentrations that would provide ten-fold dilutions from 300,000 to 30 gene copies per PCR reaction. Duplex real-time PCR reactions were performed, following the protocol described above, using 5 μl of template DNA. Three replicates of each standard curve were performed. Quality control strains were used as follows: ATCC 13565 (*sea*); ATCC 14458 (*seb*); ATCC 19096 (*sec*); ATCC 23235 (*sed*). All quality control strains were *S. aureus* and therefore carried the *fem*B gene.

2.2. Method application (Field Study)

2.2.1. Sample population

Households were recruited as a systematic subsample (homes of all adult participants with asthma able to be scheduled for home visit attendance by sub-study personnel between January 2014 and December 2015) from a larger-cohort study, "The effect of air quality on the adult asthmatic response (INHALE)." Home dust was collected from participant bedrooms during the enrollment home visit prior to intervention. Homes were recruited irrespective of housing type, number of occupants, or pet-keeping.

2.2.2. Dust collection for qPCR from homes

The participant's bed and (if needed) bedroom floor dust was collected using a study vacuum fitted with a DUSTSTREAM® adaptor and filter (Indoor Biotechnologies, Charlottesville, USA). The target quantity was one half the filter, regardless of the time required to collect the sample. The total surface area and number of surfaces vacuumed were recorded. The study vacuum was disinfected using quaternary ammonium-based disinfectant wipes between home visits. The adaptor was washed in a deionized water and dilute detergent mixture in a sonicator, air-dried and disinfected with wipes as described above. Raw dust samples from the bed and bedroom floors of asthmatic adults were divided into aliquots: 50 mg for direct DNA isolation and 150 mg for extraction processing before DNA isolation (see below). If