2. In conclusion, Hev b 12 seems to be a clinically relevant allergen in patients with an nsLTP allergy, although further studies are needed in larger study populations and additional geographic areas.

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The findings and the conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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Intracellular residency of *Staphylococcus aureus* within mast cells in nasal polyps: A novel observation

To the Editor:

Chronic rhinosinusitis (CRS) with or without nasal polyps (NPs) (CRSwNP and CRSsNP, respectively) is one of the most common conditions encountered in medicine.[1] CRS is a disease of the mucosa lining the sinonasal cavity characterized by recurrent episodes of inflammation resulting in chronic symptoms such as nasal obstruction, facial pain, rhinorrhea, and reduction in sense of smell.[1] CRS affects up to 15% of the general population in Europe and the United States, ranking it second in prevalence among chronic conditions,[1] and significantly affecting quality of life and health care resources.[2] Despite the massive expenditure on medical and surgical therapies for this condition, a subset of patients remains resistant to all established treatments.[2] Identification of either a host or an environmental cause has been unsuccessful. Proposed mechanisms of CRS pathophysiology include the role of superantigens, abnormal cell-mediated immune responses, changes in the inflammatory cytokine cascade, epithelial defects, osteitis of the sinus walls, and viral, bacterial, and fungal factors.[1] Recent guidelines propose the classification of CRSwNP and CRSsNP into 2 distinct pathological entities primarily based on differences in inflammatory cytokine profiles.[1] A CRSwNP T_H2-mediated profile is characterized as eosinophilic with elevated IL-5, IgE, RANTES, and eotaxin,[1] and a CRSsNP T_H1-mediated profile is characterized as neutrophilic with elevated TNF-α, IL-8, and IFN-γ.[1]

Emerging evidence implicates bacterial biofilms as mediators of the inflammatory reaction in CRS.[1] Several studies provide strong evidence that bacterial biofilms perpetuate inflammation in CRS and are associated with more severe preoperative disease, persistence of ongoing mucosal inflammation, and poor postsurgical outcomes.[3] *Staphylococcus aureus* has been identified as the commonest biofilm-forming microbe in CRS and colonizes the sinonasal cavities in 27% of the patients with CRSsNP and in 60% of the patients with CRSwNP.[4]

Around 20% of the patients with CRS develop NPs,[1] and although studies have clearly identified bacterial biofilms on the sinonasal mucosa of patients with CRS, little data pertain to the bacterial profiles specifically in NPs. We conducted a preliminary study characterizing bacterial profiles in NPs, comparing them with those on nonpolypoidal sinonasal mucosa from the same patients and with non-CRS sinonasal mucosa as control tissue.

A prospective study with full ethical approval was conducted in 9 patients with CRSwNP undergoing functional endoscopic sinus surgery and 5 control patients undergoing transsphenoidal pituitary surgery. Nonpolypoidal sinonasal mucosa and NPs were collected from each patient with CRSwNP and sinonasal mucosa from controls. The bacterial profiles were assessed using fluorescence *in situ* hybridization with confocal laser scanning microscopy (CLSM) and immunohistochemistry. Hybridization conditions were optimized for CRS tissue using appropriate controls (see the Methods section in this article's Online Repository at www.jacionline.org).

CLSM demonstrated surface-related bacterial biofilms on the nonpolypoidal sinonasal mucosa of all 9 CRS samples (Fig 1, *A*), but not on the epithelial surface of NPs. However, subepithelial and intracellular bacteria were observed in the cytoplasm of host cells in all 9 NP samples (Fig 1, *B*). The CLSM Z-axis view indicated that intracellular bacteria were subepithelial in all cases (Fig 1, *C*). Bacteria were confirmed within host cells using 4′,6-diamidino-2-phenylindole, a nucleic acid stain that resolves the host cell nucleus, surrounded by densely packed bacteria filling the cytoplasm (Fig 1, *D*). Biofilms were not observed in any control samples. Species-specific fluorescence *in situ* hybridization identified brightly fluorescent bacteria in CRS samples including *S aureus* in 78%, *Haemophilus influenza* in 33%, and *Pseudomonas aeruginosa* in 33% of the patients

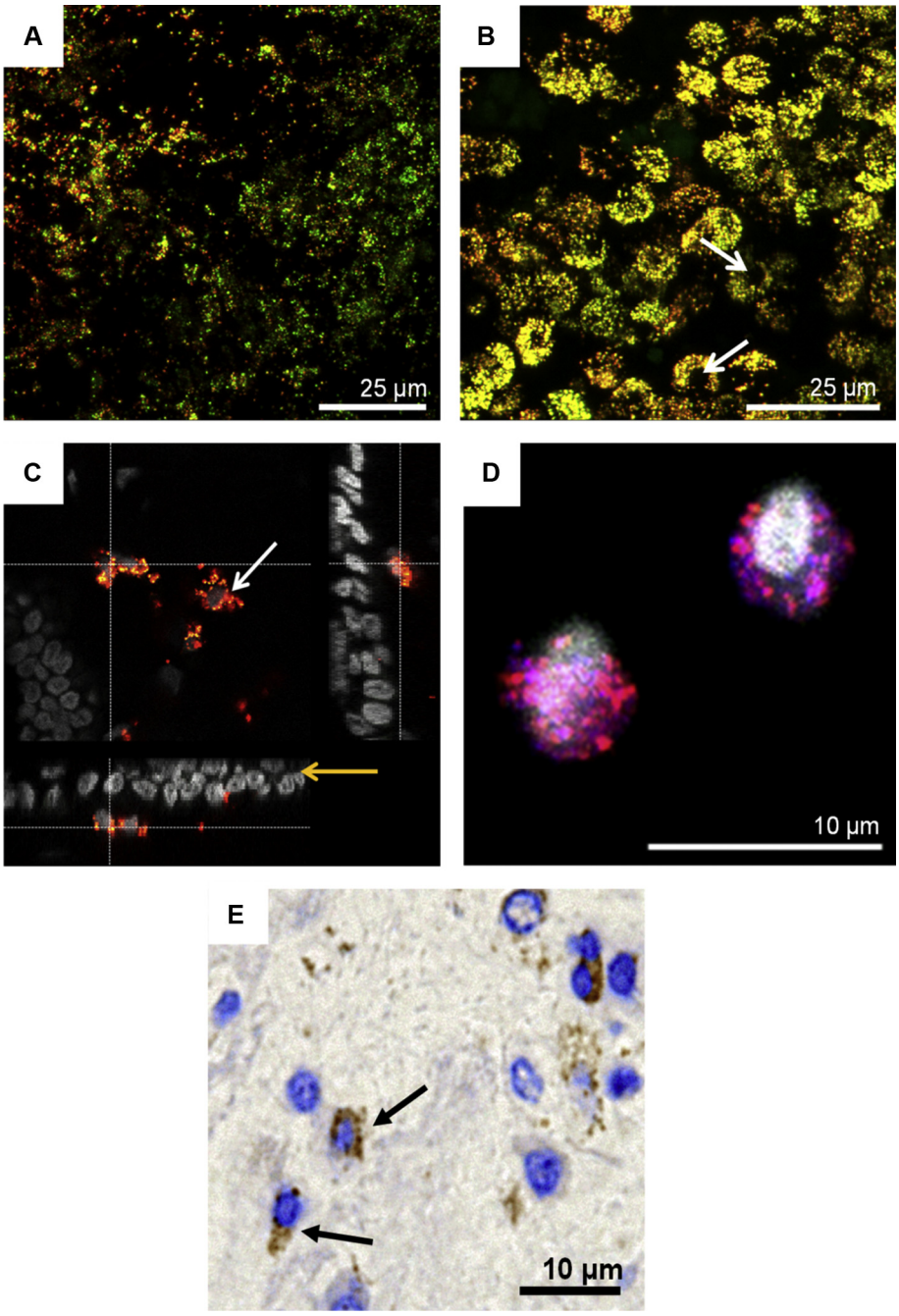

FIG 1. Intracellular *S aureus* in *ex vivo* NPs. Representative CLSM images of sinus mucosa and NP tissue following FISH showing *Staphylococcus aureus (yellow)*, *Staphylococcus* genus biofilms *(red)*, and bacteria hybridized only with a eubacterial probe *(green)*. **A,** Aggregated bacteria are widely distributed over the epithelial surface of sinus mucosa. **B,** In contrast, bacterial reservoirs surround host cell nuclei in NP biopsies *(white arrows)*. **C,** XYZ view: staphylococcal aggregates *(white arrow)* clearly visible beneath the epithelial surface *(yellow arrow)*. **D,** Intracellular *S aureus* reservoirs *(pink)* colocalized with DAPI-stained host nuclei *(gray)*. **E,** Immunohistochemistry staining with mouse anti–*S aureus (brown)* in an NP biopsy section demonstrating *S aureus* within the cytoplasm of host cells *(black arrows)* (×40 magnification). *DAPI*, 4′-6-Diamidino-2-phenylindole, dihydrochloride; *FISH*, fluorescence *in situ* hybridization.

(see Table E1 in this article's Online Repository at www.jacionline.org). Immunohistochemistry confirmed our finding of subepithelial intracellular *S aureus*, using an *S aureus* mAb (Fig 1, *E*). Immunohistochemical colocalization on sequential sections identified the *S aureus*–harboring host cells as mast cells (MCs) (Fig 2, *A-D*).

Our study directly compared nonpolypoidal sinonasal mucosa with NPs from the same patient (thus controlling for host genetics) and is the first to observe both subepithelial bacteria in *ex vivo* NP tissue and intracellular localization of *S aureus* within MCs. This contrasts with recent studies that have shown intracellular *S aureus* in nonpolypoidal CRS sinonasal mucosa.[5]