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Microbial contamination of musical wind instruments

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Retrospective and prospective studies were used to assess the numbers, types and persistence of microbes that contaminate wind instruments. All previously played instruments ($n = 20$) harbored viable bacteria as well as mold and/or yeast. Reed instruments consistently carried higher microbial loads than did flutes or trumpets. Instruments played within the previous three days bore typical mouth flora, while bacteria recovered after 72 h following play consisted of normal environmental flora. Prospective studies tested survival of potentially pathogenic bacteria (*Staphylococcus*, *Streptococcus*, *Moraxella*, *Escherichia coli* and attenuated *Mycobacterium tuberculosis*) when applied to reeds or following simulated 'play' of a clarinet. All species survived for a maximum of 24–48 h on reeds, except *Mycobacterium*, which persisted through 13 days. In simulated play experiments, test bacteria could persist for up to five days. These findings support the establishment of guidelines for decontamination of wind instruments and for sharing or transfer of these instruments among players.

Keywords: musical wind instrument; microbial contamination; infection transmission

Introduction

Few data are available on the survival of bacteria from wind instruments. However, the potential for re-contamination of players by their own instruments or cross-contamination of oral and pulmonary microbes among players who share such instruments is real. Any woodwind or brasswind instrumentalist is aware of the salivary saturation of these horns after multiple minutes of play, frequently resulting in the dripping of, or need to shake or blow out excess condensate on the floor. With repeated playing, quantities of organic matter can quickly build up inside the mouthpieces and tubing of instruments that are not regularly cleaned. Many players, particularly those in popular music performance, are prone to elevating their instruments above mouth level, which can result in backward drainage through the mouthpiece. Furthermore, some playing techniques require regular aspiration with strong, rapid bursts, which also clears the mouthpiece of excessive, interfering saliva build-up. These practices present the potential for drawing in aerosols and condensate from the instrument interior. Exposure through open keyholes and instrument disassembly also poses potential contamination for hands, although this

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is yet unexplored. A thorough discussion of instrument user practices, potential for contamination, and useful cleaning solutions is offered by Woolnough-King (1994).

Medical and dental professionals are keenly aware of the many diseases spread by saliva and blood from the oral cavity and lungs (Lewis et al. 1992; Schuster 1999). While advanced HIV patients will experience severe periodontal breakdown with ''bleeding gums,'' dentists see otherwise healthy patients who complain of nocturnal gum bleeding. Logic leads to the conclusion that musicians whose gingival tissues bleed upon slight provocation would likely introduce blood elements into the mouthpiece and body of the musical instrument which they play. Meningococcal disease and its spread via saliva and transmission of gram negative respiratory pathogens among immune-compromised patients are just two examples of the potential health problems that could arise from shared instruments (Jackson et al. 1995; Moore 2004). Recurrent sore throats and chronic airway inflammation have also been implicated empirically in the persistent reuse of wind instruments that are not regularly cleaned (Woolnough-King 1994; Tønseth 2002).

Hundreds of thousands of musical instruments exchange hands every year in elementary schools, through instrument brokers and via internet sales, both domestic and worldwide. Sharing of borrowed instruments among elementary and secondary schools is routine, particularly with larger school-owned instruments such as tubas, baritones and French horns (Babin 1992; Criswell 2009). Presently, there is no standard protocol for sanitizing a used musical wind instrument and there are no government regulations in the U.S. mandating even minimal efforts to disinfect or clean instruments between users. Measures to ensure hygiene, if any are used at all, remain entirely the prerogative of the individual school, private owner, or borrower. The recent MRSA (methicillin-resistant *Staphylococcus aureus*) outbreaks and the rise of the HIV and H1N1 swine influenza epidemics have heightened awareness of community hygiene measures, especially among sports and exercise enthusiasts, but little or no attention has been devoted to wind instrument players. Because there are few published reports on the microbial contamination of musical instruments and on the potential transfer of pathogenic bacteria from instrumentalist to instrument and vice verse (Protheroe 1957; Walter and Chaffey 1959; Osterholm et al. 1979; Tønseth 2002; Moore 2004) there are minimal valid data available to support and guide intervention.

In the research described here, both retrospective and prospective studies were undertaken to determine the numbers and types of microbes associated with wind instruments. To our knowledge, these experiments provide the first prospective data on survival of bacterial pathogens in wind instruments.

Materials and methods

Instrument testing

For the retrospective studies (Figure 1), instruments were obtained from a commercial instrument broker and also from private owners who played their instruments on a regular basis. Through the use of latex gloves (to avoid skin flora contamination), instruments were disassembled into their component parts and sterile cotton-tipped applicators pre-moistened in 1 ml of iced, sterile buffered saline were used to thoroughly swab the surfaces as described in Figure 1. Applicator swabs were mixed vigorously by vortexing for 30 sec in 1 ml saline, and the expressed suspension was maintained on ice. Ten-fold dilutions in saline were made and plated

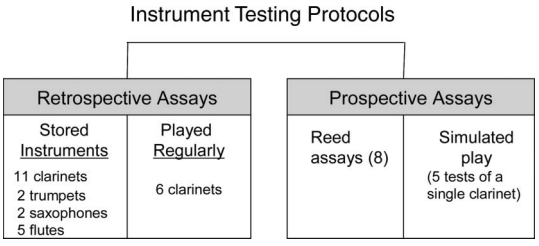

Figure 1. Experimental design for testing wind instruments. To test for bacterial survival in wind instruments, both retrospective and prospective assays were undertaken. Retrospective assays consisted of testing stored or recently played instruments that had not been cleaned. For clarinets and saxophones, samples were taken by swabbing the entire surface of each of the following: internal and external mouthpiece, the internal neck (clarinet only), body, bell, the insides of keyholes and key pad material. For the flute, the mouthpiece and inside surfaces of the upper and lower body were tested. For trumpets ($n = 2$) 5 ml of saline was passed through the instrument and the recovered amount ($\sim$1.4 ml) was used to swab the bell, shaft and valves for additional bacteria. Of these two, one contained 10^4 mold, while both contained 10^2–10^3 environmental-type cfu.

by an EddyJet spiral plater (IUL Instruments, Barcelona, Spain) onto media for the isolation of aerobic bacteria: trypticase soy agar (TSA) for total colony-forming units (cfu); TSA 5% blood agar for the identification of hemolytic bacteria; and MacConkey agar for the recovery of gram-negative bacteria. When available, clarinet reeds (natural, ''Giant Cane'') were tested by vortex mixing in 3 ml of buffered saline for 30 sec and plating serial dilutions of the suspension as described above. Plates were incubated 24–48 h at 35°C and with 5% CO_2 for all blood agar.

Bacterial strains and isolation procedures

Viability studies that examined the persistence of selected bacterial species were undertaken following deliberate inoculation (prospective analyses) of the reed itself, or by aerosolization of a saliva suspension of bacteria through a clarinet. Wild-type bacterial strains used for these assays were: *Staphylococcus aureus* (Elaine Larson, Columbia University, NY, USA) *Streptococcus pyogenes, Moraxella catarrhalis* (Paratek Pharmaceuticals, Boston, MA, USA) and *Escherichia coli* (human fecal isolate SLH25, our laboratory). Colonies grown overnight on blood agar plates were suspended in saline to a turbidity of MacFarland 2–3. BCG (attenuated *Mycobacterium* tuberculosis; William Jacobs, Albert Einstein College of Medicine, NY, USA) was grown in nine parts Bacto Middlebrook 7H9 medium (Difco) supplemented with one part volume of filter-sterilized albumin dextrose complex (ADC) enrichment (per 100 ml: glucose 2 g, bovine serum albumin fraction V powder 5 g, NaCl 0.85 g).

Reed inoculation

Saline-moistened natural reeds (steam-sterilized for BCG experiments) were suspended in 2 ml of freshly collected saliva to which an estimated 10^5–10^6 cfu of test bacteria had been added and agitated vigorously (Vortex, Genie 2) for 30 sec. For certain experiments (i.e., testing optimal recovery and BCG), the saliva was partially or completely sterilized by low-level microwave exposure (sub-boiling

temperature, determined experimentally) to reduce indigenous competing bacteria to $\leq$10 cfu/ml. Each inoculated reed was then removed and suspended vertically in a loosely capped, plastic 15 ml conical centrifuge tube (BD Falcon) and incubated at 25°C. A control reed was removed at time zero for determining the inoculum count. At select time points, a reed was removed for vortexing (30 sec) in 2 ml of sterile buffered saline before plating. All plates were incubated at 35°C in 5% CO_2 for 48 h. The phenotype patterns on blood agar were classified as alpha-hemolytic, beta-hemolytic and gamma-hemolytic. The selected strains were visually distinguishable from mouth flora as follows: *S. aureus* – large yellow, beta-hemolytic; *S. pyogenes* – small beta-hemolytic off-white, M. *catarrhalis* small, gamma-hemolytic, off-white with characteristic "hockey-puck" movement when pushed. Mouth flora consisted of alpha- or gamma-hemolytic phenotypes.

Simulated play

A clarinet was selected for these experiments for several reasons: (1) Clarinets demonstrated high levels of contamination in our retrospective analyses, (2) they are one of the commonest small instruments used, and (3) they also have a straight body with internal surfaces that can be readily accessed with swabs as well as multiple open keyholes for sampling. In order to disperse bacteria throughout all portions of the instrument as would occur during normal play, a 1-jet Collison nebulizer (BGI, Waltham, MA, USA) which contained a suspension of sterile saline (40 ml) + saliva (2 ml, sterilized as described above) + test bacteria (5×10^4 – 5×10^6 cfu) (maintained at 37°C to mimic body temperature) was joined to the mouthpiece by means of a tubular ring of latex rubber (cut from a surgical glove) and clamped to form an airtight seal. The nebulizer was connected to a positive air pressure source via latex tubing attached to a pump (deVilBiss Co, Somerset, PA, USA) and a steady air pressure was supplied and regulated to deliver 20 psi (Matheson Instruments flowmeter, Horsham, PA, USA) per manufacturer's recommendations. The aerosolized mixture was blown through the mouthpiece for 30 min, which resulted in dispersal throughout the clarinet. The instrument was then capped and stored at room temperature for testing at defined time points as described above.

Results

Retrospective studies

A total of 20 previously played wind instruments (11 clarinets, five flutes, two saxophones and two trumpets [mouthpieces absent]) were tested for the presence of microbial flora. All instruments showed some level of viable bacteria, mold or yeast, regardless of when they had last been played (Figure 2). With one exception, all instruments held in long-term storage (one month or longer) exhibited only gamma-hemolytic flora. In contrast, those played within the previous three days showed abundant alpha-hemolytic bacteria indicative of mouth flora (presumptive *Streptococcus* spp) (Schuster 1999). These latter organisms became a useful marker for the persistence of commensal mouth flora in the instruments.

The numbers of viable colony forming units (cfu) varied widely from instrument to instrument. Those carrying an attached reed and showing much accumulated debris tended to present high recoveries (up to 300 million bacteria) (Figure 2), and frequently, large numbers of mold and/or yeast. The highest numbers were typically

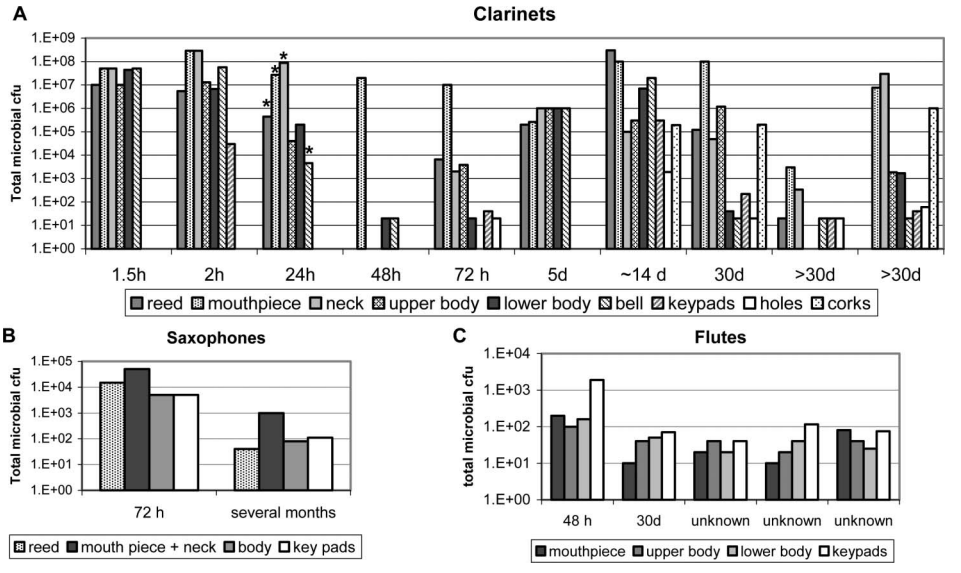

Figure 2. Retrospective recovery of bacteria on previously played instruments. At various time points following play, clarinets (A), saxophones (B), and flutes (C) were disassembled and their components were swabbed in their entirety for total microbial counts. Trumpets (not shown) carried <5000 bacteria and mold cfu in their internal pipes. Three flutes were retrieved from storage and had not been played for an undefined time (>1 month). *indicates recovery of alpha hemolytic "mouth flora". None was observed at ≤2 h, presumably because of overgrowth of other species that obscured this phenotype.

found on the upper portions of the instrument, specifically the reed, the mouthpiece and neck, with markedly decreasing cfu found on lower portions (Figure 2). Reed-bearing instruments (clarinets, saxophones) consistently produced higher counts (Figure 2A, 2B) than non-reed instruments (flutes, trumpets), which carried relatively few bacteria (Figure 2C). New reeds carried few bacteria (<10 cfu), whereas used, stored reeds sometimes exceeded 5×10^4 cfu (data not shown). Corks and keypads contained organism phenotypes which were similar to those recovered in other instrument parts. Yeasts and molds frequently predominated, particularly with longer-term storage.

Prospective studies

Since retrospective experiments showed that the great majority of organisms recovered were confined to the upper instrument parts, i.e., the mouthpiece, and more specifically the reed, experiments were undertaken to test survival outcomes in two types of prospective studies: (i) The inoculation of reeds stored within a confined (humid) environment at room temperature, and (ii) the inoculation of a reed instrument during simulated play.

In a total of eight reed experiments, inocula of 5×10^6 mouth bacteria from saliva showed survivals ranging from 24–74 h post inoculation. No alpha-hemolytic mouth flora were recovered at 96 h; therefore, it is concluded that the maximum survival time for these classic mouth flora on a stored reed under non-drying conditions would be between three and four days. Survival of inoculated pathogens

Table 1. Maximum pathogen survival on reeds and clarinets*.

Strain	On reeds	Clarinets in simulated play
Streptococcus pyogenes	24 h	24 h
Moraxella catarrhalis	<24 h	20 h
Streptococcus pneumoniae	<24 h	<48 h
E. coli	2 days	3 days
Staphylococcus aureus	2 days	2–5 days**
BCG	13 days†	ND
hemolytic mouth flora	3 days	3 days

*Strains were inoculated onto reeds or aerosolized through clarinets via a nebulizer. Recovery was assessed as described in the methods; **For the longer survival time, the instrument was stored enclosed within an instrument case; †Not tested beyond 13 days.

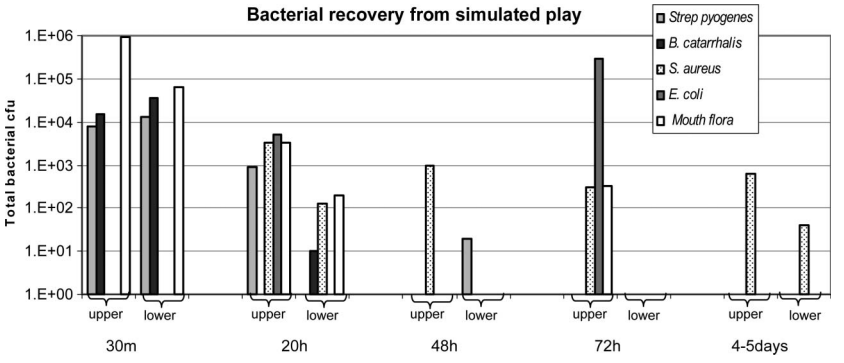

Figure 3. Recovery of test bacteria from the simulated playing of a clarinet. Each bar represents a simulation experiment from which the combined bacterial counts of the "upper" three components (mouthpiece, reed and neck) or the lower three components (upper body, lower body, and bell) were assessed. No *Streptococcus pneumoniae* (tested at only 48 h post play) were detected. *E. coli* was not tested at 30 min.

varied with the species tested and ranged from less than 24 h (*Moraxella*, *Streptococcus*) up through 13 days (BCG) (Table 1). In simulated play experiments, 10^4–10^6 test bacteria could be found within 30 min of use throughout most instrument parts. Approximately 10^3 cfu could still be recovered from the upper portions 24 h later. A general pattern of decline in recovery over time was observed; however, $\sim 10^5$ *E. coli* could be recovered 72 h after simulated play and 10^2–10^3 *S. aureus* persisted up through 4–5 days (Table 1, Figure 3). Under these experimental conditions, bacterial recoveries never exceeded the original inocula.

Discussion

The question of contaminated wind instruments as sources of infection is not new and dates back in the literature to the 1950s (Walter and Chaffey 1959). All subsequent studies examined the presence of bacteria associated with played instruments. In these studies we undertook prospective as well as retrospective examinations for bacterial persistence on the surfaces of musical wind instruments.

Clarinets and saxophones were selected to represent those instruments in which the player forcibly blows lung air into a tubular form, past a wooden reed. Flutes and trumpets represented those in which air was blown through a small aperture, typically metal.

Organisms representing mouth flora or classic pathogens were not recovered from instruments that had been played more than 72 h previously. However, large numbers of microbial organisms typically considered to be normal environmental flora common to soil, air, and water were recovered from stored instruments, particularly from the mouthpieces and reeds (Figure 2). In an independent unpublished examination of used wind instruments, Anderson Products [Laboratory Services Division] determined that these organisms constituted primarily the following genera: *Sporosarcina*, *Planococcus*, *Azotobacter, Micrococcus* spp. *Acidomonas*, *Acetobacter* and occasionally the commensal skin species *Staphylococcus caseolyticus* and *epidermidis* (personal communication). In his unpublished report on the survey of instruments actively played by music students, Woolnough-King (1994) identified beta-hemolytic *Streptococcus* spp and the following species of *Staphylococcus*: *S. capitus*, *S. cohnii*, *S. epidermidis*, *S. hominus* and *S. saprophyticus*. In a retrospective examination of horn mouthpieces, Bryan (1960) reported gross contamination with multiple microorganisms, but did not define the species involved.

Survival of prospectively applied test bacteria (representing potential pathogens or pathogen surrogates), varied with the species and persisted for as long as five days post inoculation and at least 13 days for the *Mycobacterium* strain BCG. This finding is consistent with other reports which found that survival of test bacteria (*Streptococcus salivarius*, *Staphylococcus* and *E. coli*) on plastic, wood and paper surfaces was strain and moisture dependent, but could persist for hours to several days (1–3.5 days) when dried in water, saliva, saline or broth suspensions (Pettit and Lowbury 1968; Marshall et al. 1988; Tagg and Ragland 1991). Other studies have demonstrated survival of *Staphylococcus aureus* from 1–90 days after drying on a variety of gas-sterilized fabrics and polyethylene plastic (Neely and Maley 2000). *Streptococcus pyogenes* has remained viable for up through 20 weeks when dried in human blood on paper toweling (Reitmeyer et al. 1993). The more limited pathogen recovery in our retrospective experiments may relate to competition from other environmental flora, including yeasts and fungi, which tended to increase in the instrument biofilm with extended storage. The finding of extended survival of mycobacteria ($>$13 days) supports a previous report in which high numbers of several opportunistic *Mycobacterium* spp. were recovered from a single horn (Ademollo 1974).

Our prospective study results represent survival in a single clarinet which was repeatedly swabbed and therefore did not contain detritus build-up. As epithelial debris, food particles and saliva provide nutrients, these elements could impact further on survival.

The finding of high levels of microbial contamination concentrated in the upper mouthpiece and on reeds (Figures 2 and 3) suggests the potential for bacterial cross-contamination from sharing instruments, or from reinoculation of a player through repetitive playing. The infective dose for pathogens is variable, being dependent on many factors, including host health, immunity and skin trauma. It is reportedly as small as 10 viable cells for certain pathogenic *E. coli* and for *Mycobacterium tuberculosis* and from 10^4–10^6 cells for *S. aureus* and *S. pneumoniae* (Schmid-Hempel

and Frank 2007). However, the extent to which persons actually reinfect themselves or others with reuse or sharing of their instruments is not known.

Reports of direct links between playing wind instruments and acquiring an infectious disease are rare (Protheroe 1957; Tønseth 2002). In a study of the dynamics of TB transmission among the closed community of a military band that rehearsed ~30 h per week in a small unventilated room, Protheroe (1957) concluded that the constant drip of condensate and saliva on the floor, with subsequent inhalation of aerosolized dust, was partly responsible for inter-player infection. While, theoretically, wind instruments could serve as fomites for bacterial and viral transmission between players, empirically the evidence for this route of transmission for HIV, herpes simplex, Hepatitis B and Epstein Barr is weak (Babin 1992). Osterholm et al. (1979) examined inadvertent exposure to hepatitis B virus through shared use of an instrument between students and their music instructor. While the authors concluded that sharing of the uncleaned instrument offered a high potential for spread, there was no evidence for transmission of hepatitis B to the students. The negative finding may have related to the possible absence of hepatitis B antigen in saliva at the time of transmission, or the known low infectivity of hepatitis B through saliva. Babin (1992) suggested that the greater infection risk lies with transmission of cold or influenza viruses through shared instruments and to those sitting in front of the bells of brass or horn instruments. Studies of this transmission route have not been undertaken; however, our experiments confirmed that mouth bacteria could penetrate clarinets and persist in high numbers in the lower end of the instrument for at least 30 min. For wind instrumentalists, the risk of infection from classic bacterial pathogens via inter-person sharing and intra-person reinfection appears greatest during the first 72 h following play. These data generally concur with those reported for influenza A and B, which survived for 24–72 h depending on surface type (Bean et al. 1982; Boone 2007). Concern has also been expressed that wind instruments may contain opportunistic pathogens that pose a risk through repetitive playing by the same person (Bryan 1960; Ademollo 1974; Tønseth 2002; Nieminen et al. 2003; Moore 2004). Probable reinfection of a healthy instrumentalist from continued playing of a horn contaminated with a highly resistant *Pseudomonas* spp has been reported. Treatment with antibiotics and consistent weekly cleaning of the horn resolved the chronic illness (Tønseth 2002). More recently there has been an increasing development and application of music therapy for the palliative management of certain diseases, including asthma and chronic obstructive pulmonary disease, especially among children (Moore 2004). These and other patients with AIDS or immune-compromised conditions are more susceptible to the opportunistic infections from common environmental microbes. Thus while the risk from inter-person sharing appears to decline after ~72 h, the exposure to fungi and yeast contamination appears to increase, thereby elevating the possibilities for pulmonary irritation and infection from repetitive play.

Because our primary focus was on bacterial pathogens, these studies were not designed to accurately recover and assess mold and yeast contamination or viruses. However, it is evident that research in this area as it relates to chronic pulmonary infection is needed. Interestingly, asthma is the most common chronic pulmonary disorder among wind instrumentalists (Gilbert 1998). This group also exhibits reduced pulmonary function, but the exact reasons for this remain unclear (Deniz et al. 2006). For those who suffer recurrent airway infections, instrument hygiene should be investigated as a causative factor.

In recent years, outbreaks of bacterial meningitis have been traced to customary, but unhygienic practices within certain sports in which team members share the same water bottles (Zangwill et al. 1997). Guidelines have warned against such practices, but these have not necessarily been extended to safeguard against pathogen transfer among those sharing musical instruments. The traditional method in which most wind instruments are maintained and stored cannot be considered ''hygienic'' (Legnani et al. 2004) and has been equated with ''storing used silverware until the next meal without cleaning'' (Walter and Chaffey 1959). Visible films of detritis were often evident within the mouthpieces of the reed instruments that we tested. Even if an instrument receives a cursory dry-cloth wiping, moisture will remain in the reeds, crevices and tubing, and the instrument (including any pull-through swab or cleaning cloth), is typically sealed in its instrument case with no exposure to circulating air. Over time, these conditions tend to perpetrate mold growth, as was found in instruments stored for 30 or more days.

Conclusions

Walter and Chaffey (1959) found the combination of brushing and rinsing mouthpieces to be more effective than simple wiping or rinsing with plain water in lowering the numbers of residual organisms after playing. The implementation of a combination brush/sanitizer tank apparatus for horn mouthpiece disinfection in a high-school band room decreased contamination approximately 1000-fold, particularly with successive post-use treatments (to $\sim$10–100 cfu) (Walter and Chaffey 1959). Nonetheless, recommendations for improving instrument hygiene and discouraging infection transmission (Bryan 1960; APMT 2010) have not been widely adopted. The modest, but accumulating evidence that potentially harmful bacteria can persist for significant periods of time on musical wind instruments suggests that greater precautions should be exercised in the recycling of instruments through schools, sales and rental shops. A synthesis of safe practices from the literature includes the following recommendations: (1) All instrumentalists should have their own instrument and mouthpiece and their own personal reeds; (2) where single ownership is not possible, i.e., for instruments that must be shared in a class or demonstration situation, alcohol wipes or soap and water should be utilized for hand and instrument disinfection between different players; and (3) instruments that are returned for recycling through schools or commercial outfits should be disassembled and cleaned with a commercial disinfectant, a 1:10 solution of chlorine bleach and water (if appropriate for the instrument), alcohol, or soap and water. Recently, commercial ethylene gas sterilization of instruments has also become available for this purpose (www.maestromd.com). We would also suggest that swab pull-throughs and other drying cloths could be regularly microwaved to speed up drying and to decrease contamination. Reed decontamination by this method may be effective, but could result in premature deterioration of natural reeds (Casadonte 1995). Importantly, all students should be taught how to clean their own instruments to keep them hygienic (Babin 1992).

More research is needed on the transmission risks of both viruses and bacteria among wind instrumental groups. The long-term risks posed by chronic exposure to opportunistic pathogens in instrument biofilms also merit further investigation and implementation of appropriate public health recommendations for reducing these risks.

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