

Evaluation of the microbial flora found in woodwind and brass instruments and their potential to transmit diseases

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Previous studies of dental devices (toothbrushes, dentures, and protective athletic mouthguards) have demonstrated microbial contamination of these devices and possible transmission of infectious diseases to the users. Since woodwind and brass instruments come into intimate contact with the musician's oral cavity and often are passed from student to student without sanitization, the question arises as to whether these instruments are contaminated and can transmit microbial diseases. The purpose of this study was to determine if woodwind and brass instruments and/or their cases harbor opportunistic, pathogenic, or allergenic microorganisms that can be transmitted to the musician.

The internal components of woodwind and brass instruments

harbored opportunistic, pathogenic, and/or allergenic microorganisms. The highest concentrations of microorganisms were found consistently at the mouthpiece end, but there was evidence of contamination throughout the instruments and their cases. The close proximity of contaminated mouthpieces to the oral cavity could facilitate local and systemic dissemination of the resident opportunistic, pathogenic, and/or allergenic microorganisms. General dentists should determine whether patients play a brass or woodwind instrument and be aware of the possible impact of this activity on the oral cavity and the entire body.

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Many children and young people participate in school and extracurricular band ensembles. Woodwind and brass instruments comprise a substantial portion of these ensembles. Often, instruments used by students are on loan from the school and previously have been played by individuals whose health histories are unknown to the recipients. Also, private organizations, such as the Mr. Holland's Opus Foundation, distribute donated used instruments to underprivileged inner-city children.¹ Used woodwind and brass instruments have not been evaluated thoroughly as a suitable habitat for microbial growth. However, the mouthpieces, internal tubing, intricate valves, keys, pads, hinges, and cases could provide potential sites for microbial contamination, facilitating the transmission of microbial diseases.

When various parts of woodwind and brass instruments are used,

they become repositories for the users' oral and pulmonary secretions.² Because these instruments come into intimate contact with the musicians' oral and respiratory mucous membranes, such exposures may facilitate microbial transmission. Furthermore, as these instruments are repeatedly played, they build up visible amounts of organic material, providing an excellent habitat for microbial growth. Even though the instruments may lie dormant during the summer months between school sessions, they could remain contaminated.

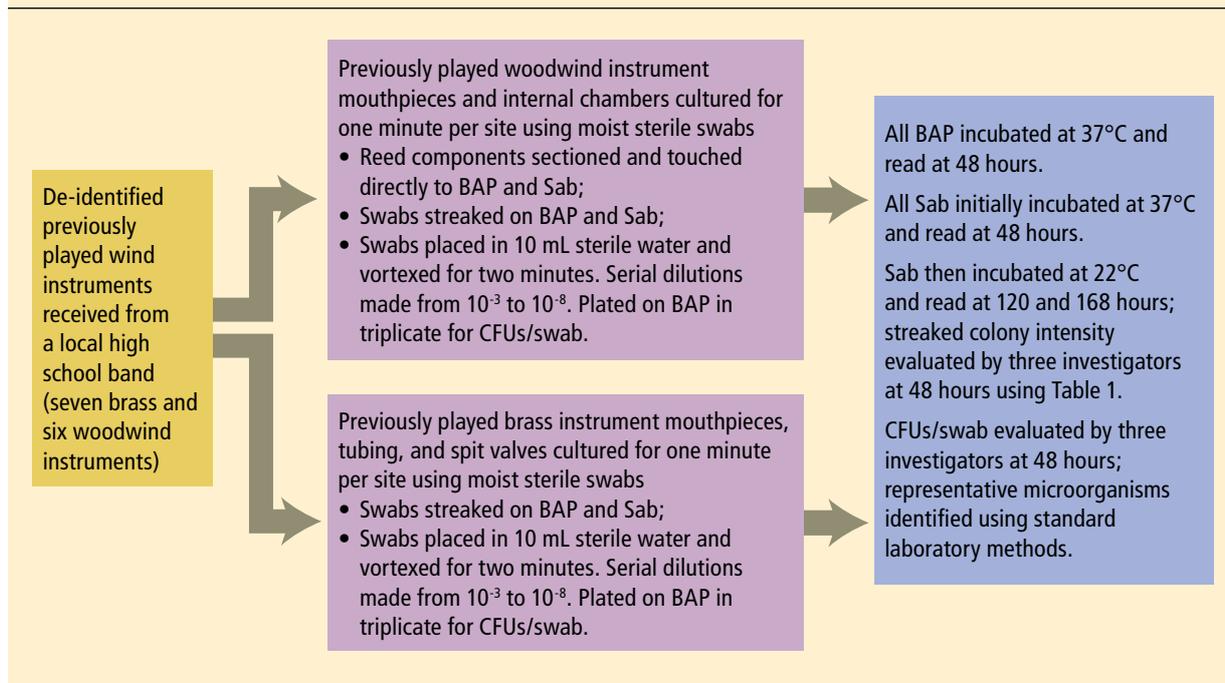
Methods for clearing such organic accumulations from woodwind and brass instruments include repeatedly aspirating the secretions from the instrument; evacuating materials from water valves (spit-traps); wiping areas with fingers and cleaning cloths; and flushing the instruments with antimicrobial solutions. These routine procedures

could provide further opportunities for disease transmission by contaminating the musicians' hands, which in turn could contaminate other instruments or the musicians' eyes, nose, or mouth. In addition to direct contact, microorganisms could be expelled into the local enclosed environment (the band room) by playing the instruments.

There has been a great deal of research recently into the transmission of microorganisms, including bacteria, fungi, and viruses by oral means. However, little is known regarding the specific health hazards associated with the sharing of contaminated wind instruments. Despite studies confirming a relationship between breathing difficulties and playing wind instruments, no association has been made with the effects of instrument contamination.^{3,4}

While minimal research has been conducted specifically on microorganisms harbored in wind

Chart 1. A flow chart outlining the experimental design of this study.



instruments, there is an extensive body of research regarding the presence of opportunistic and pathogenic microorganisms on and within oral devices. Multiple studies conducted by Glass *et al* found that toothbrushes harbor pathogenic microorganisms involved in oral, pulmonary, and systemic diseases.⁵⁻¹³ These researchers also noted that patients who are immunocompromised were at far greater risk than healthy individuals of developing microbial diseases through contaminated toothbrushes.^{9,12}

Additional research on other oral devices such as dentures and protective athletic mouthguards found colonization by similar potential disease-producing microorganisms.¹⁴⁻²³ In the most recent study of 53 protective athletic mouthguards, Glass *et al* found that the most common of the 253 Gram-positive isolates were *Staphylococcus spp.* (182) and *Micrococcus spp.* (54).²⁴

The remaining 17 isolates were various *Streptococcus spp.* In the same study, 14 of the *Staphylococcus* isolates were *S. aureus*, two of which were methicillin-resistant (MRSA). Of the non-aureus *Staphylococcus spp.*, 53% were methicillin-resistant, while 76% of the *Micrococcus spp.* were methicillin-resistant. Even more disconcerting was the finding that 71% of the non-aureus *Staphylococcus* and *Micrococcus spp.* were resistant to triclosan, a common antimicrobial agent used in some instrument rinses. This finding of resistance factors in non-aureus *Staphylococcus spp.* has important clinical implications for the prevention and treatment of infections in humans.

A 2009 study of 1,391 hospitalized patients found that 188 (13.5%) had antimicrobial-resistant infections (ARI), resulting in medical costs ranging from \$18,588–\$29,069 per patient.²⁵

The additional societal costs in this cohort were estimated to be \$10.7–\$15.0 million. Based on this study and several others, it is suggested that such avoidable infections resulted in more than \$35 billion in societal cost annually and more than 8 million additional days spent in the hospital nationwide.²⁶

The hypothesis of this study was that the internal components and/or the cases of woodwind and brass instruments harbor potentially pathogenic, opportunistic, or allergenic microorganisms that can be isolated and identified by routine laboratory methods.

Materials and methods

In order to answer the hypothesis, the protocol was followed as outlined in Chart 1. A local small-town high school band agreed to participate in the proposed study. For this institutional review board (IRB)-approved study, 13 de-identified

Table 1. Colony intensity scale.

Value	Colonies/cm ²
0	≤5
1	6–25
2	26–100
3	>100, but without confluent growth
4	too numerous to count, with confluent growth

previously played wind instruments (seven brass and six woodwinds) were utilized. Although the instruments were de-identified, a history was obtained regarding the length of time between when the instrument was last played and the testing. Six of these instruments (three brass and three woodwinds) had been played within a week of testing, while the other seven instruments (four brass and three woodwinds) had not been played for at least one month prior to the study.

Before the microbial flora were sampled, appropriate photographs were made of the interstices and cases of the instruments. A total of 117 different sites, including the mouthpieces, internal chambers, and cases of the study instruments, were cultured by swabbing each area with a moist sterile swab for one minute. All swabs were immediately streaked onto a blood agar plate (BAP) and a Sabouraud dextrose plate (Sab). The reeds were touched directly onto the media, both intact and after cross-sectioning. The swabs or reeds were then placed in 10 mL of sterile water and vortexed for two minutes. Serial dilutions of the test waters were made from 10⁻³ to 10⁻⁸ and plated on BAP in triplicate for enumeration of colony-forming units (CFUs)/swab. The BAP cultures were incubated

Table 2. The most commonly isolated bacteria (occurring in at least three instruments), with their Gram stain results and illnesses they could produce.

Species	Gram stain/morphology	No. of instruments (n = 13)	Potential diseases
<i>Aureobacterium spp.</i>	Positive/bacilli	5	Systemic infections in immunocompromised patients
<i>Bacillus cereus</i>	Positive/bacilli	5	Diarrheal/emetic toxins; septicemia; bacteremia; ocular virulence; osteomyelitis
<i>Bacillus megaterium</i>	Positive/bacilli	5	Food poisoning; cerebral abscesses
<i>Brevibacterium spp.</i>	Positive/bacilli	11	Corneal infections; food poisoning; endophthalmitis
<i>Burkholderia cepacia</i>	Negative/bacilli	6	Pulmonary pathogen for patients with cystic fibrosis, skin and soft tissue infections, surgical wound infections, and genitourinary tract infections
<i>Cellulomonas spp.</i>	Positive/bacilli	7	Acute cholecystitis; sepsis; infective endocarditis; osteomyelitis
<i>Chryseobacterium luteola</i>	Negative/bacilli	8	<i>Pseudomonas</i> -like, opportunistic pathogen; high drug resistance
<i>Kocuria varians</i>	Positive/cocci	9	Brain abscess; opportunistic pathogen in immunocompromised patients
<i>Micrococcus spp.</i>	Positive/cocci	9	Opportunistic pathogens in immunocompromised patients; drug-resistant
<i>Staphylococcus capitis</i>	Positive/cocci	7	Septicemia; endocarditis; catheter-related infections
<i>Staphylococcus epidermidis</i>	Positive/cocci	6	Nosocomial infections; wound infections; postsurgical infections
<i>Staphylococcus hominis</i>	Positive/cocci	3	Septicemia; blood cultures
<i>Staphylococcus lentus</i>	Positive/cocci	3	Arthritis; urinary/catheter/prosthetic joint infections
<i>Staphylococcus saprophyticus</i>	Positive/cocci	3	Female urinary infections
Other <i>Staphylococcus spp.</i>	Positive/cocci	10	Arthritis; catheter and prosthetic joint infections; urinary tract infections.

at 37°C and were read at 24 and 48 hours. The Sab were incubated initially at 37°C and read at 48 hours for yeasts, then incubated at 22°C and read at 120 and 168 hours for molds. The CFUs/swab were evaluated and tabulated at 48 hours by three investigators. All BAP- and Sab-streaked plates were scored

at 48 hours, using the previously described colony intensity scale shown in Table 1.^{14,16-19,21}

Bacteria and yeasts were identified using standard laboratory methods, including Gram stains and API strips (bioMerieux, Inc.). Molds were identified and preliminary yeast identities were confirmed

using standard molecular techniques (DNA analyses).

Given the numbers of microorganisms and limited funds/time, antibiotic susceptibilities were performed on only Gram-positive cocci. The susceptibility procedures used standard antibiotic-impregnated disks on pure culture lawns of microorganisms (Kirby-Bauer test).^{27,28} The drugs tested were penicillin, oxacillin (methicillin), vancomycin, ciprofloxin, tetracycline, erythromycin, gentamicin, and azithromycin. Zones of microbial inhibition were measured and compared to standards for determination of susceptibility/resistance to individual antibiotics.

The data were analyzed statistically. Correlation coefficients (R^2) were determined using correlation and regression analyses. The p values were determined using the unpaired Student's t -test.

Results

A total of 117 sites were sampled on 13 de-identified instruments, which consisted of two clarinets, two oboes, two saxophones, two mellophones, two trombones, two trumpets, and one cornet. The most frequently isolated bacteria (occurring in three or more instruments) are listed in Table 2, while the most frequently isolated fungi (occurring in three or more instruments) are listed in Table 3. Examples of partial instrument analyses (random sites) are demonstrated in Figures 1 and 2.

A total of 442 bacterial isolates were initially identified. After eliminating redundancies, 295 different isolates were found in the 117 test sites, for an average of 2.5 isolates/site. Based on colony appearance, morphology, Gram stain reaction, and biochemical means (API strips), the 295 isolates consisted of 95 (32.2%) Gram-positive cocci, 131 (44.4%) Gram-positive bacilli,

Table 3. The most commonly isolated fungi (occurring in at least three instruments), with their type and the illnesses they could produce. Note that many of the fungi are associated with allergic diseases and that one, *Fusarium oxysporum*, produces a mycotoxin.

Species	No. of instruments (n = 13)	Type	Pathogenicity
<i>Aspergillus niger</i>	4	mold	opportunistic
<i>Aureobasidium pullulans</i>	3	yeast	allergenic; opportunistic
<i>Bipolaris spp.</i>	3	mold	allergenic; opportunistic
<i>Candida albicans</i>	3	yeast	opportunistic
<i>Cochliobolus spp.</i>	6	mold	allergenic; opportunistic
<i>Cryptococcus laurentii</i>	4	yeast	opportunistic
<i>Fusarium oxysporum</i>	7	mold	allergenic; mycotoxin; opportunistic
<i>Paecilomyces lilacinus</i>	5	mold	allergenic; opportunistic
<i>Penicillium chrysogenum</i>	11	mold	allergenic; opportunistic
<i>Rhodotorula mucilaginosa</i>	7	yeast	allergenic; opportunistic

and 69 (23.4%) Gram-negative bacilli; no Gram-negative cocci were isolated. Of note, only one instrument was positive for *Staphylococcus aureus*. Many of these bacterial isolates are considered to be frank or opportunistic pathogens.

All Gram-positive cocci (comprising 14 different species) were tested against a battery of antimicrobials, including methicillin. High levels of methicillin resistance were detected in isolates of *Staphylococcus aureus* as well as in other *Staphylococcus spp.* Furthermore, similar levels of methicillin resistance were found in Gram-positive cocci that are generally considered to be non-pathogenic. Methicillin resistance did not correlate with resistance to any other antimicrobial tested against the Gram-positive cocci.

Using standard laboratory and molecular techniques, a total of 19 yeast isolates were detected in eight of the 12 instruments (all six woodwinds and both mellophones). All of the identified yeasts could be

considered as opportunistic and/or allergenic pathogens.

The 13 instruments also yielded a total of 58 molds. Again, all of the mold isolates could be considered as opportunistic and/or allergenic pathogens. Interestingly, seven of the mold isolates (*Fusarium oxysporum*) are potential mycotoxin producers. Mycotoxins are secondary metabolites (byproducts) of the growth of the molds and can have substantial toxic side effects for plants, animals, and humans. Certain mycotoxins are also considered carcinogenic.²⁹

Even though the number of instruments (13) was low, 117 individual sites were available for statistical analyses. The statistical analyses of the data revealed the following findings:

- There was a high level of correlation between the two methods of quantification (touch culture evaluations compared to serial dilution with colony counts) ($R^2 = 0.9442$).

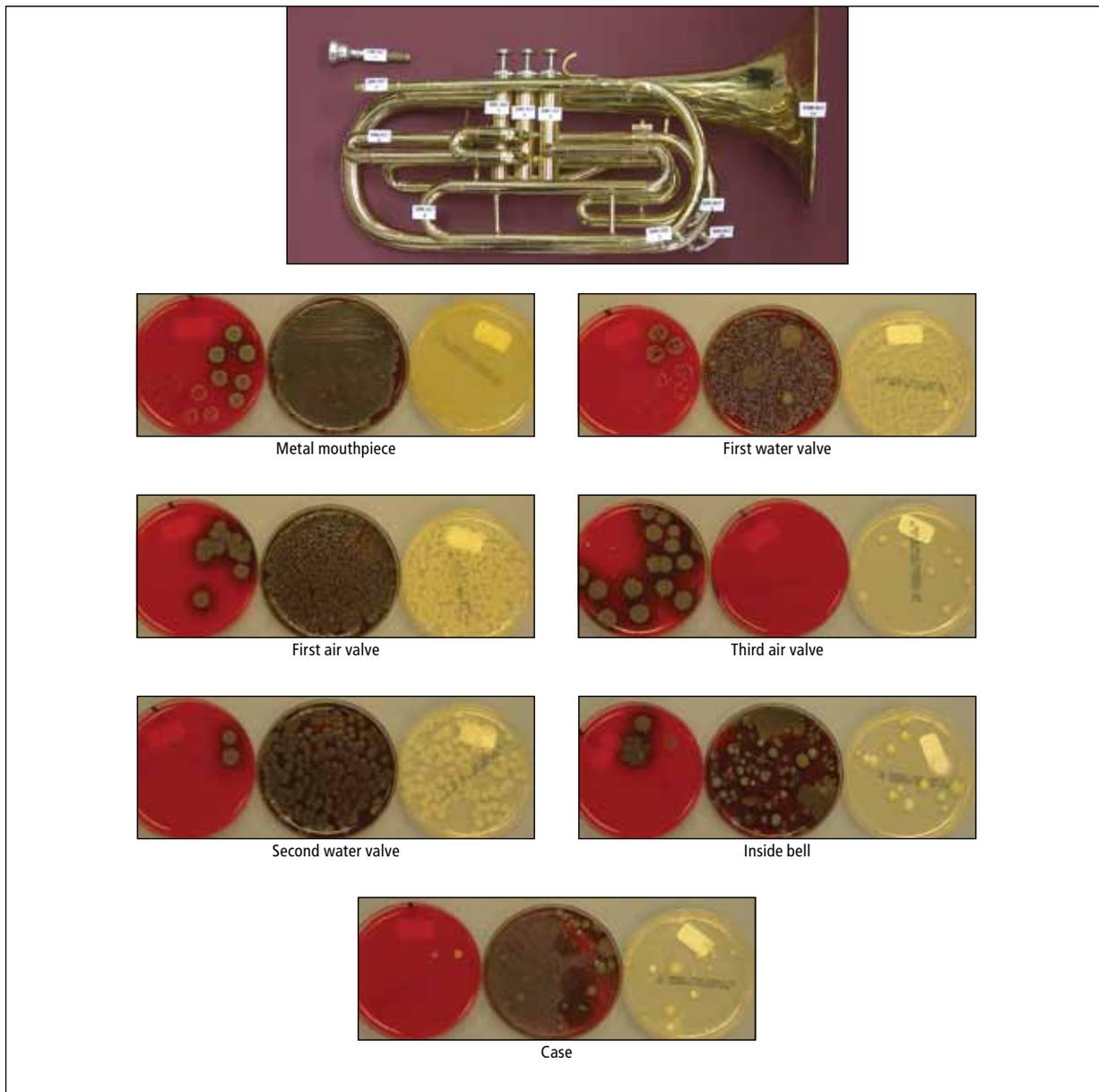


Fig. 1. An example of cultures taken from representative sites of a mellophone. The plate on the left shows the CFUs/swab on BAP; the plate in the middle shows the swab streak on BAP; and the plate on the right shows the swab streak on Sab. Note the quantitative and qualitative differences from site to site, including the instrument case.

- There was a low level of correlation between the last time an instrument was played and the bacterial load, confirming that some bacteria remained viable in instruments that had not been played in more than a month ($R^2 = 0.1520$).
- There was an intermediate level of correlation between contamination in the reed/mouthpiece (the most proximal site), the instrument midpoint, and the bell (the most distant site) ($R^2 = 0.856$). The reed/mouthpiece ends were consistently more contaminated than the bell ends. However, it should be noted that

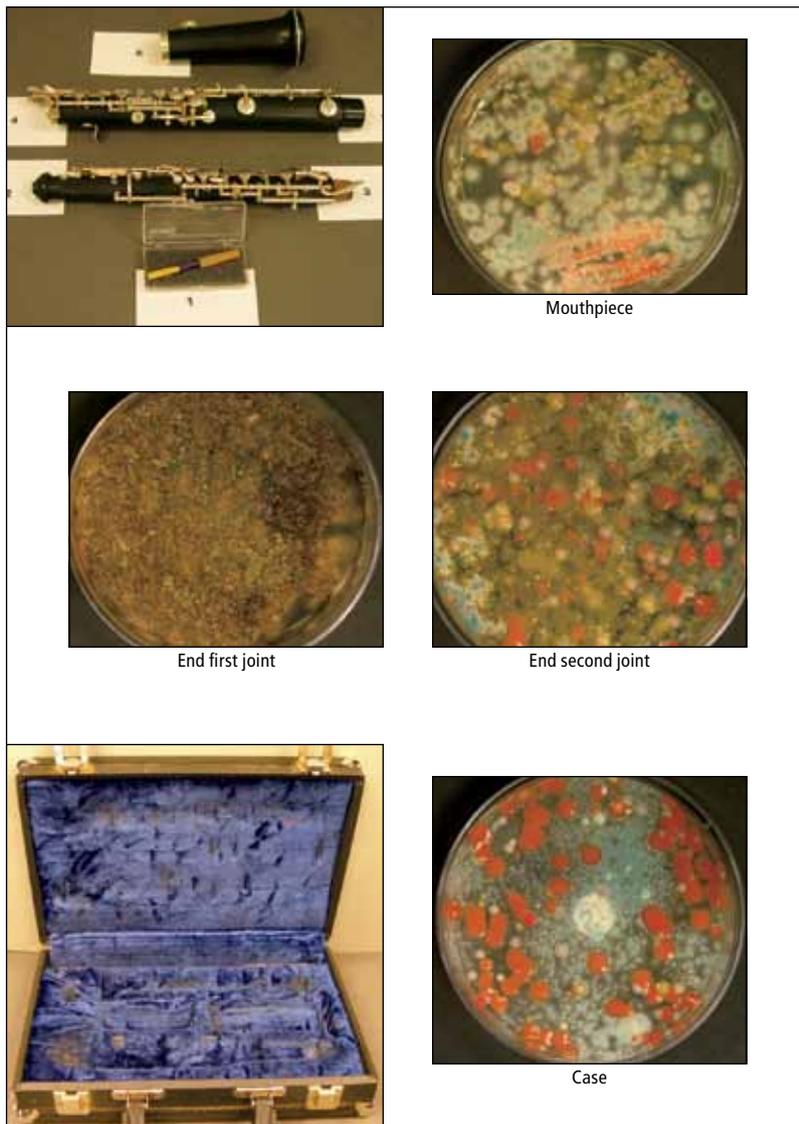


Fig. 2. An example of fungal streak cultures taken from representative sites of an oboe. Again, note the quantitative and qualitative differences from site to site, including the instrument case.

both the instrument midpoints and bells retained microorganisms in sufficient quantities to affect transmission, expose the musicians to toxins, and produce disease.

- Analyses of the differences between the bacterial loads in woodwinds and brass instruments yielded a p value of 0.1547, suggesting that woodwind

instruments were more heavily contaminated than brass instruments.

- Analyses of the differences between the bacterial loads in reeds as compared to mouthpieces yielded a p value of 0.0496, indicating that reeds were significantly more heavily contaminated than mouthpieces.

- Analyses of the differences between the bacterial loads in clarinets and other woodwinds yielded a p value of 0.0479, indicating that clarinets were significantly more heavily contaminated than other woodwinds.
- Analyses of the differences between the bacterial loads in clarinets and all other instruments yielded a p value of 0.0026, indicating that clarinets were significantly more heavily contaminated than all other instruments, including brass.
- Analyses of the differences between the bacterial loads in metal instruments (including saxophones) and wood/plastic instruments (clarinets and oboes) yielded a p value of 0.2376, confirming that the composition of the instrument did not affect contamination.
- Analyses of the differences between the bacterial loads in trombones and all other instruments yielded a p value of 0.2229, confirming that contamination of trombones was not statistically different from that of other instruments.
- Analyses of the differences between the bacterial loads in the bells of the instruments and the cases yielded a p value of 0.6864, confirming that both sites were equally contaminated.
- Analyses of the differences between the bacterial loads in the mouthpieces and the cases yielded a p value of 0.0131, confirming that the mouthpieces were significantly more heavily contaminated than the cases.
- Analyses of the differences between the bacterial loads in the reeds and the cases yielded a p value of 0.0043, confirming that the reeds were significantly more heavily contaminated than the cases.
- Analyses of the differences between the bacterial loads in woodwind instrument cases and

brass instrument cases yielded a *p* value of 0.0008, confirming that not only were the woodwind instruments significantly more heavily contaminated than brass instruments, their cases were, too.

Discussion

The purpose of the present study was to determine whether wind instruments are contaminated by either frank or opportunistic pathogenic microorganisms, which can cause significant disease in the person playing the instrument. Such results could be useful in determining whether these microbes posed a danger of a significant magnitude to warrant periodically sterilizing the instrument to ensure the safety of the musician.

The study followed Chart 1 and measured microbial intensity by both visual examination and CFUs/swab. As confirmed by statistical analyses of the data, there was a statistically significant positive correlation between the two methods of evaluating microbial load.

The results of the current study confirmed that wind instruments are heavily contaminated with a wide variety of bacterial and fungal isolates. Identification of these microbes down to the species level was completed; however, the authors wish to note that using these standard laboratory methods did not isolate fastidious pathogens such as spirochetes, mycoplasma, mycobacteria, and viruses.

The results of the current study also indicate that wind instruments are contaminated with a number of potentially harmful microbes, many of which are associated with minor to serious infectious or allergic diseases. Furthermore, this study also found that many of these microbes are highly resistant to some or most of the antibiotics normally used in general practice, including methicillin.

The medical literature is replete with examples of carriers such as “Typhoid Mary” who harbor and spread potentially deadly diseases without suffering ill effects themselves. The results of this study found that wind instruments could act as reservoirs of such diseases. For this reason, prudence demands that the presence of actual or opportunistic pathogens must be taken seriously in order to protect susceptible musicians from these microorganisms.

It must be stressed that while the results found the heaviest contamination in the reed/mouthpiece sites, there were sufficient microorganisms throughout the instrument interstices and cases to warrant regular sterilization of the entire instrument. Another unexpected finding was that the species of microorganisms were not consistent throughout the instruments. In other words, the microorganisms isolated from the sites closer to the mouthpiece end were different from those isolated from sites closer to the bell end.

The current study confirmed the hypothesis that the internal components of woodwind and brass instruments and their cases harbor potentially pathogenic, opportunistic, and/or allergenic microorganisms. The study also confirmed that microorganisms can be isolated from various components throughout instruments and their cases and can be identified by routine laboratory methods. Because most of the microorganisms detected in this study are considered pathogenic, opportunistic, and/or allergenic, sterilization of the instrument is recommended on a routine basis, and definitely before an instrument is passed to a new user. Currently, ethylene oxide is the only agent known to sterilize instruments effectively.³⁰

Because this study used de-identified instruments, no medical histories were obtained. However, anecdotal information from the band teacher/leader confirmed that, at any given time, more than 50% of the band students had some respiratory distress (asthma or bronchitis) that required therapy. Therefore, additional studies must be performed to determine the microbial concentration in the band room before, during, and after band practice. In addition, demographic and medical histories need to be obtained from each band member to confirm the anecdotal information obtained from the band teacher/leader. Finally, because this study analyzed wind instruments obtained from a rural setting, a comparable study should be performed in an urban environment to compare findings.

Conclusion

The results of this study revealed that wind instruments and their cases become contaminated with use and that this contamination can last for extended periods of time. Many of the bacterial and fungal isolates must be considered to be pathogenic, opportunistic, and/or allergenic pathogens. In addition, this study validated the methods used to study contamination of wind instruments and their cases.

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