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Original Article

A SERIES OF SIMPLE DECONTAMINATION METHODS OF BACTERIAL FLORA FOUND ON MUSICAL WIND INSTRUMENTS

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ABSTRACT

Objective: This study was aimed to compare the efficacy of cleaning techniques using hot water treatment soap containing 2% triclosan and chemical antiseptics in reducing the bacterial contamination observed on shared musical wind instruments.

Methods: The trumpet, mellophone, trombone, and tuba were evaluated in this study. To count the initial bacterial colonies on the instrument, the total amount of bacteria adhered to it was extracted using the swab procedure. The mouthpieces were immersed in hot water at a temperature of 100 °C for 5 min and then were soaked in soap that contained 2% triclosan to achieve the effect of decontamination. Then the survival colonies were counted. As a series of decontamination technique, this study also examined the disinfection ability of phenol, chloroxylenol, povidone-iodine, and 70% alcohol utilizing the Rideal Walker method.

Results: When compared to liquid soap (50.30-91.67%), the cleaning procedure that uses immersion in hot water of 100 °C for 5 min greatly lowers the quantity of bacteria (91.85-99.91%). However, due to their huge surface area, tuba mouthpieces were the most straightforward to sterilize using both techniques. The highest phenol coefficient value was shown by chloroxylenol; however, all tested disinfectants showed stronger antibacterial activity than 1% phenol.

Conclusion: The mouthpieces of shared wind instruments can be cleaned quickly, easily, and effectively by immersing them in hot water at a temperature of 100 °C for 5 min. However, chloroxylenol has the strongest ability to eradicate bacteria from the instrument's mouthpiece.

Keywords: Mouthpieces, Musical, Cleaning, Chloroxylenol, Hot water

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INTRODUCTION

While certain infections may be able to survive without a living host in a dormant condition, many pathogens need a living host to survive. However, in order to spread from one host to another, all diseases need mechanisms. It is essential to comprehend how infectious microorganisms propagate to inhibit the spread of infectious diseases. The transmission of pathogens between hosts via fomites plays a significant role in the spread of infectious illnesses [1-3]. Numerous studies have examined particular facets of music-making, particularly when using wind instruments, to evaluate potential transmission concerns. For particular instrument groupings and instruments, results were occasionally a little bit different. Risk profiles for various instruments were indicated by contrasting the release of particles during speaking and breathing with the release during music creation [4, 5]. However, playing notes that were particularly loud and brassy was not linked to a specific enhanced particle emission [6]. As an illustration, wind instruments may contain thousands of harmful organisms. These devices involve possible risks in the transmission of infections if multiple players use the same one. Numerous wind instruments' mouthpieces have been found to contain millions of bacteria when they are kept or after use [7]. According to some research, salivary bacteria could adhere to mouthpieces and subsequently be blown deeper into wind instruments. Additionally, because those wind instruments are typically on loan, other people may have utilized them. According to reports, the woodwind and brass instruments were infected with a variety of microbial flora linked to infectious and allergic illnesses [8]. Particularly interesting is a fatal incidence of hypersensitivity pneumonitis in a bagpiper. Likewise, gastrointestinal anthrax in an animal-hide percussionist serves as a reminder of this uncommon yet seriously dangerous illness [9, 10].

Staphylococcus, Escherichia coli, Streptococcus, Moraxella, and attenuated *Mycobacterium tuberculosis* survived when applied to reeds in a simulated investigation involving multiple harmful microorganisms. As a result, it showed that the levels of contamination had significantly increased in the time immediately following play. All bacterial species, with the exception of Mycobacterium, remained on reeds for a maximum of 24–48 h [11]. Therefore, bacteria and fungus might continue to grow for weeks or even months after the last usage if thorough cleaning or disinfection is not performed. If the instrument is shared or purchased, it should be disassembled and cleaned with alcohol wipes, soap and water, or a commercial disinfectant in order to limit the spread of germs. Due to the sensitivity of bacteria to high temperatures, 100 °C hot water is frequently used to destroy bacteria. By lowering the surface tension of bacterial cells, washing soap can kill bacteria [12]. To reduce pollutants, soaps and other cleansing agents have been used for a very long time. The purpose of soap is to reduce the inoculum levels of bacteria, both pathogenic and non-pathogenic. Attention has been drawn to the use of antiseptics in the mouthpiece as a serial decontamination technique to lower pollutants. Therefore, in this study, we explore an easy-to-use cleaning technique so that the owner of the wind instruments can thoroughly clean and sanitize the instruments. Combine with the various disinfection methods currently being utilized to maintain the mouthpieces of wind instruments from disease transmission.

MATERIALS AND METHODS

Samples

Instruments were collected from a marching band's wind located in Jatinangor, West Java, Indonesia, as well as from private owners who regularly played their instruments. The trumpet, mellophone, trombone, and tuba, were studied and employed as sources for bacterial isolates. The mouthpiece samples used were previously played mouthpieces.

Bacterial isolation

Instruments were disassembled into their component pieces using latex gloves (to prevent skin flora contamination), and sterile cotton-tipped applicators were pre-moistened in 1 ml of cooled water. By swabbing the inside of the mouthpieces with a sterile cotton swab then thoroughly mixed by vortexing in 10 ml of saline

for 30 s, and the expressed solution was kept chilled. To isolate aerobic bacteria, six-fold dilutions in saline were prepared, plated using a hockey spreader onto trypticase soy agar (TSA) media, incubated at 37 °C for 18 h, and total colony-forming units before treatment were counted (cfu) [11].

Physical decontamination

Hot water physical disinfection and soap with 2% triclosan were contrasted. A wind instrument's mouthpiece is submerged in hot water heated to 100 °C for 5 min. Then, for the agar plate count, the suspended bacteria were diluted (10^{-4}, 10^{-5}, and 10^{-6}). The amount of bacteria was then computed in order to determine how many bacteria would survive treatment. Wind instrument mouthpieces are treated by submerging them in water with soap that contains 2% triclosan and treated as the hot water method [13].

Chemical decontamination

In order to chemically decontaminate musical instruments, the phenol coefficient and contact time testing of phenol, chloroxylenol, povidone-iodine, and 70% alcohol were performed using a Rideal walker method. Isolated bacteria from mouthpieces and other instruments were the test organisms. This culture was kept alive by a subculture on TSA medium, which was incubated for 24 h at 37 °C before being placed in a refrigerator and kept at or below 22 °C. A small amount of the most recent subculture's growth was transferred to a sterile tube containing TS Broth medium and cultured for 23 h at 37 °C as part of this study. After that, a Standard loopful was moved to a different tube and incubated there as before. Before conducting a test, this was done at least three times. In sterile distilled water, stock solutions of standard phenol (used as a standard) and disinfectants (used as a test sample) were created. The stock solution was then used to make the appropriate dilutions in sterile distilled water [13].

Suspension of bacteria

All isolated bacterial strains from nutrient agar slants were transferred as a loopful culture into 10 ml of nutrient broth and cultured for 20 to 24 h at 37 °C. In normal saline, tenfold serial dilutions were performed. The amount of cfu/ml was determined by plating 0.1 ml from each dilution onto the nutrient agar petri plates, spreading it evenly, and incubating the plates at 37 °C for 24 h. The number of colonies that developed was multiplied by the dilution factor to determine the number of cfu/ml.

RESULTS

There are few data on bacterial survival in wind instruments. However, the potential for recontamination of players with their own instruments, or cross-contamination of oral and pulmonary microbes between players sharing such instruments, is real [11]. This study examined four mouthpieces of wind instruments: the trumpet, mellophone, trombone, and tuba. The number of germs present in each mouthpiece for each treatment was calculated based on bacteria on the mouthpieces. Only plates with fewer than 250–300 bacterial colonies can often be counted [12]. Table 1 illustrates how cleaning procedures reduce the amount of germs that survive. The cleansing method using immersion in hot water with a temperature of 100 °C for 5 min significantly decreasing the bacteria number (91.85-99.91 %), compared with that of liquid soap (50.30-91.67 %). The percentage of colonies reduction was the highest in the disinfection of trombone mouthpieces.

Table 1: Percentage of colony decrease

Instrument	Cleansing treatment	Initial cfu/ml	Difference of decrease	Reduction (%)
Trumpet	0	$5.73.\ 10^{5}$±0.00	-	-
	1	$4.67.\ 10^{4}$±0.00	$5.263.\ 10^{5}$±0.00	91.85±0.00
	2	$6.7.\ 10^{3}$±0.00	$4.\ 10^{4}$±0.00	85.65±0.00
Mellophone	0	$2.027.\ 10^{6}$±0.00	-	-
	1	$0.4.\ 10^{5}$±0.00	$1.987.\ 10^{6}$±0.00	98.03±0.00
	2	$6.7.\ 10^{3}$±0.00	$3.33.\ 10^{4}$±0.00	71.31±0.00
Trombone	0	$7.2.\ 10^{6}$±0.00	-	-
	1	$6.7.\ 10^{3}$±0.00	$7.1933.\ 10^{7}$±0.00	99.91±0.00
	2	$3.33.\ 10^{3}$±0.00	$3.37.\ 10^{3}$±0.00	50.30±0.00
Tuba	0	$23.06.\ 10^{6}$±0.00	-	-
	1	$0.4.\ 10^{5}$±0.00	$2.302.\ 10^{7}$±0.00	99.83±0.00
	2	$3.33.\ 10^{3}$±0.00	$3.667.\ 10^{4}$±0.00	91.67±0.00

Notes: n=triple replication; 0 treatment= without treatment; 1= treatment using hot water at a temperature of 100 °C for 5 min; 2= treatment using 2% soap containing triclosan

Tables 2 and 3 show the results of the phenol coefficient test to ascertain the concentration of the quickest and longest disinfectant test to destroy isolated bacteria. The value of the phenol coefficient is then calculated using the concentration as can be seen in table 4. According to the results of the phenol coefficient test, the tested antiseptics had a phenol coefficient value greater than 1. This means that the tested antiseptics had a greater capacity for killing than 1% phenol. The disinfectant's killing ability increases with the test disinfectant phenol coefficient value.

Table 2: The fastest bactericidal concentration of the tested disinfectants (2.5 min)

Instrument	Concentration (%v/v)			
	Phenol	Chloroxylenol	70% alcohol	Povidone Iodine
Trumpet	1	0.5	0.5	0.75
Mellophone	0.75	0.5	0.75	0.75
Trombone	0.75	0.25	0.75	0.5
Tuba	1	0.5	0.5	0.5

Table 3: The longest bactericidal concentration of the tested disinfectants (15 min)

Instrument	Concentration (%v/v)			
	Phenol	Chloroxylenol	70% alcohol	Povidone Iodine
Trumpet	0.5	0.125	0.25	0.5
Mellophone	0.5	0.125	0.25	0.5
Trombone	0.25	0.125	0.25	0.25
Tuba	0.25	0.125	0.25	0.25

Table 4: Phenol coefficient value

Instrument	Phenol coefficient value		
	Chloroxylenol	70% alcohol	Povidone iodine
Trumpet	3.00	2.00	1.16
Mellophone	2.75	1.50	1.00
Trombone	2.50	1.00	1.25
Tuba	2.00	1.50	1.50

DISCUSSION

In order to better comprehend the threats to the public's health posed by musical wind instruments, a number of additional factors also need to be taken into account. Due of the customary means by which student musicians obtain their instruments, the usage of wind instruments in public schools is a subject of particular concern. In most cases, students (or their parents) buy their instruments from local music retail shops or from music dealers connected to their school. However, some of them can be acquired through "rent-to-buy" plans provided by school music retailers. On the persistence of germs from wind instruments, little information is available. However, there is a significant risk that players could become re-contaminated by their own equipment or that players who share such equipment could become cross-contaminated with oral and pulmonary microorganisms. Any woodwind or brass wind musician is aware that these horns become salivary saturated after a few minutes of use, frequently causing in the need to shake or blow off extra condensate on the floor. In instruments that are not routinely cleaned, large amounts of organic debris can quickly accumulate inside the mouthpieces and tubes with repeated playing. Many musicians, especially those who perform popular music, have a tendency to hold their instruments above lip level, which can cause rearward leakage via the mouthpiece. Additionally, some playing approaches call for frequent aspiration in quick, powerful bursts that also clean the mouthpiece of excessive, disruptive saliva buildup. These procedures run the risk of bringing condensate and aerosols from the inside of the instrument [11]. The possibility of bacterial cross-contamination from sharing instruments or from reinoculating a player through repetitive playing is suggested by the discovery of significant levels of microbial contamination localized in the upper mouthpiece and on reeds [14]. Sharing and borrowed instruments is common practice between elementary and secondary schools, especially when it comes to heavier instruments like tubas. Instruments that had been played more than 72 h prior to the recovery did not contain any organisms that represented mouth flora or conventional infections. However, a lot of microbes that are generally thought of as part of the regular flora of soil, air, and water were found in abundance in the mouthpieces and reeds of stored instruments [11]. However, in a separate, unpublished analysis of used wind instruments, Anderson Products [Laboratory Services Division] found that the majority of the organisms belonged to the following genera: Sporosarcina, Planococcus, Azotobacter, Micrococcus spp., Acidomonas, Acetobacter, and on occasion, the commensal skin species Staphylococcus. As possible pathogens or pathogen surrogates, the tested test microorganisms survived for up to five days after inoculation, and for at least 13 d in the case of the Mycobacterium strain BCG. This result is in line with prior studies that found that the test bacteria (*Streptococcus salivarius*, Staphylococcus, and *E. coli*) could survive on surfaces made of plastic, wood, and paper for hours to several days (1-3.5 d), depending on the strain and moisture levels [15-17]. Other investigations have shown that *Staphylococcus aureus* can survive for up to 90 d after drying on a range of fabrics that have been gas-sterilized and polyethylene plastic [18]. Therefore, the detection of bacterial presence in the mouthpieces of shared wind musical instruments among the marching bands player in Jatinangor, are very important. From our study, not only the contamination data can be informed, but also we will provide the simple decontamination method, which can be easy to be applicated by them. As the hub of the educational district in Sumedang, West Java, Indonesia, the Jatinangor marching band serves as the musical hub for a group of students from several institutions and schools. Therefore, it is essential to address the importance of disease transmission by wind instruments.

For a very long time, soaps and other cleaning products have been used as the first step to reduce contaminants. This is important to be conducted as it is reported that saliva, food particles, and epithelium debris all contain nutrients and may have additional effects on survival. Our study observed that all mouthpieces of all tested shared wins musical instruments had contaminated after 72 h used. Among the mouthpieces, our observations revealed that the tuba's mouthpiece had the highest concentration of bacterial colonies per ml. This purportedly occurred as a result of the tuba's bigger cross-sectional area than other wind instrument mouthpieces. A large cross-sectional area of the mouthpiece provides more room for bacterial development. When compared to liquid soap (50.30-91.67%), the cleaning procedure that uses immersion in hot water that is 100 °C for 5 min greatly lowers the quantity of bacteria (91.85-99.91%). The disinfection of trombone mouthpieces had the highest percentage of colonies reduced. Because bacteria are sensitive to high temperatures, 100 °C hot water is frequently used to destroy bacteria. By lowering the surface tension of bacterial cells, washing soap can kill bacteria [19].

The soap was used to reduce the inoculum sizes of bacteria, both pathogenic and non-pathogenic. When exposed to soap-containing triclosan, *Staphylococcus aureus* and *Candida albicans* displayed the zone of inhibition [13]. Triclosan (TCS) is so often used that it is probably found in consumer goods and personal care products that 75% of Americans use [20]. Recently, it was discovered that TCS decreased the variety of microbial species rather than the absolute number of microorganisms [21]. However, excessive use of antimicrobial soaps may result in the emergence of microbicide resistance. Numerous studies have claimed to demonstrate a link between triclosan use and antibiotic resistance [22-24]. Both treatments can be combined for optimal cleansing the mouthpiece of a wind instrument. The ease of cleansing methods used making the frequency of cleansing of wind instruments will be more meaningful. Musical instruments can be disinfected after a thorough cleaning process. Mouthpieces and other instruments must be carefully cleaned before use because disinfectants cannot get rid of grime. Simply by removing the filth and grease that hazardous bacteria and viruses attach to, simple soap and water can be extremely successful in reducing the amount of these organisms [25].

The cleaning process greatly increases the effectiveness of the antibacterial action of the disinfectant. Exposure period, surface properties, and organic matter are three variables that affect how effective disinfectants are. Due to its tough environment, which includes extensive areas of porous surface and a high level of organic matter, commercial poultry houses are especially hostile to disinfectants [26]. In our study, we made an effort to mimic that challenging environment without introducing the uncontrollable variables of surface features and excessive organic matter into our analysis. To achieve this, we optimized the duration of exposure and made the test setting more hospitable to the isolated bacteria. From our study, within 15 min of exposure, the majority of disinfectants suspended in clean water effectively controlled the majority of isolated bacteria from all mouthpieces. Among the tested antiseptics, Chloroxylenol has the strongest ability to eradicate microorganisms from a marching band instrument's mouthpiece. Chloroxylenol can accelerate the reduction of unfavorable levels of contamination when used in conjunction with heating and the triclosan-containing soap cleaning procedure. This conclusion can be drawn from the high potency of phenol against bacterial contamination in trumpet mouthpieces, which are more challenging to remove than mouthpieces for other wind instruments. This is most likely because the antibacterial spectrum of chloroxylenol is broader than that of the other tested disinfectants. Chloroxylenol, also known as para-

chloro-meta-xylenol (PCMX), is an antiseptic and disinfectant used to clean non-living surfaces and the skin. It can be found in household antiseptics, wound-cleansing products, and antibacterial soaps. Chloroxylenol is efficient against bacteria, fungus, algae, and viruses and displays broad-spectrum antibacterial activity. Due to its phenolic composition, halophenol has been demonstrated to be most efficient against Gram-positive bacteria, where it breaks the cell wall [27, 28]. Although the precise mechanism of action of Chloroxylenol is yet unknown, it involves interactions between the hydroxyl-OH groups of CHL and cytoplasmic membrane proteins in the cell membrane, which cause cell death and disruption [27]. The World Health Organization has listed chloroxylenol as one of the essential medicines. Meanwhile, iodophores are iodine-releasing substances that are created by mixing iodine and a solubilizer in water because only iodine is insoluble in water. Povidone-iodine, for instance, has been used for a long time as an antiseptic for different microorganisms on the skin and tissues [29-31]. The released elemental iodine has the ability to permeate membranes and damage protein's sulfuryl and disulfide linkages in addition to slowing down the aging of nucleic acids. Whereas ethanol is used as disinfectants to kill bacteria, fungus, and viruses. These disinfectants' affinities and concentrations determine their biocidal activity. 60 to 80 percent of alcohol has the best antibacterial activity [32]. Protein denaturation is the most plausible reason for alcohol's antibacterial effects. The fact that absolute ethyl alcohol, a dehydrating agent, is less bactericidal than alcohol plus water solutions supports this process since proteins denature more quickly in the presence of water [33, 34]. Protein denaturation also fits with the findings that ethyl alcohol enhances the lag phase of Enterobacter aerogenes [35] and that the lag phase impact may be reversed by adding specific amino acids. Alcohol also kills the dehydrogenases of *Escherichia coli* [36]. The generation of metabolites necessary for quick cell division was thought to be inhibited, which is what gave rise to bacteriostatic action. However, the sensitivity or resistance of various microbe types depends on a variety of microorganism kinds and environmental circumstances.

CONCLUSION

The present investigation demonstrated that there are significant differences in the disinfectant effectiveness of treatment using hot water at a temperature of 100 °C for 5 min and treatment using 2% soap containing triclosan on surfaces contaminated mouthpieces of all tested shared wind musical instruments. Among the tested antiseptics, chloroxylenol has the strongest ability to eradicate microorganisms from a marching band instrument's mouthpiece.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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