Efficacy of Traditional Medicine on Respiratory Pathogens in Association with Covid 19 Suspected Orofacial Cleft Cases

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Abstract
Cleft palate or cleft lip is an abnormal anatomical problem that causes aspiration. In India, approximately about 35,000 babies are born every year with cleft lips or palates. These anomalies seem to encourage the upper respiratory tract illness and the viral infection associated co-infection like secondary bacterial pneumonia and sepsis. Novel coronavirus, COVID 19 and its associated co infection caused by bacteria and fungi seem to be an unprecedented crisis throughout the world. Present study aimed to find the intervention of cleft palate in developing the risk of coronavirus associated bacterial pneumonia. Total of 62 samples including cleft palate as well as non-cleft were collected from patients suspected to corona reported in the medical college. The bacterial strains were characterized by morphological and biochemical methods followed by yielded strains were tested with matured fresh garlic bulbs, onion and honey for their antibacterial potential. The synergistic activity of honey-lemon mixture was analyzed. Home medicine like garlic, onion, lemon and honey showed significant anti-bacterial activity against bacterial coinfection in connection with COVID19 suspected cases. However cleft palate anomalies did not show any significant impact on such cases. Present study concludes the home medicine lemon honey mixture may reduce the risk of co-infection pneumonia and sepsis in suspected cases during acute phase of infection.

Keywords: OrofacialCleft, Home Medicine, Covid-19, Co Infection, Antibacterial Susceptibility Test

Introduction
In India, approximately about 35,000 babies are born every year with cleft lips or palates[1]. Unless it is treated properly, the craniofacial anomalies or cleft palate seem to encourage the viral infection associated co-infection like secondary bacterial pneumonia and sepsis. However, these are the...
leading cause of mortality and morbidity throughout the world[2]. After many decades, it is reported that the novel corona virus (COVID 19) has been rapidly spread across the world and it was reported initially in China. COVID 19 and its associated co-infection caused by bacteria and fungi seem to be an unprecedented havoc throughout the world [3,4]. Now a days diagnosis of covid 19 stages and its treatment seeming to be the challenging task to the doctors as well as researcher in terms of tracing the contact history of COVID 19 and treating the comorbidities in association with this disease. [5]. Indeed, aged persons with immunocompromised are more prone to be infected with COVID 19 associated coinfections[6] COVID 19 is acquired through respiratory tract, since RTI is an important site for exogenous microbes travelling and their survival then it gain access to lungs and causes lung damage. Microbiome composition of respiratory tract is individually varying according to age, earlier infection history, craniofacial anomalies, and lung disease [7]. In URT, Streptococcus pneumoniae, Neisseria meningitides, and Haemophilus influenzae are harbored as commensals. Normally healthy persons having lungs are a sterile environment, but, the cleft palate individuals with chronic airway diseases (e.g., cystic fibrosis, chronic obstructive pulmonary disease) generally develop bacterial populations in the lungs [8]. Nonetheless, bacterial colonization of the URT is made generally due to invasiveness of bacteria Comorbidities in association with S. pneumoniae infection surge the mortality rates of viral infections. However it might be resulted due to the epithelial cells damage of the respiratory tract [9]. Recent decades, many studies focusing on multi drug-resistant strain, S. pneumoniae, DRSP as it has been associated with the clinical and public health problems[10,11]. Human rhinovirus boosts the internalization of Staphylococcus aueresin to epithelial cells of RTI by releasing IL6. HC0v NL63. COVID 19 also promotes the secondary coinfections and the complications like Pneumonia, Acute respiratory distress syndrome, Sepsis, abnormal clotting and Kidney failure [12]. As cleft occurs as part of a syndrome, that may affect other parts of the body like the heart, lungs or kidneys which might increases the risk of COVID-19 [13]. To combat this clinical issue, many experts and public health organizations meticulously promulgated the strategies in developing appropriate antibiotics and vaccination for hindering the spread of DRSP. However, bacteremia has been the cause of highest risk mortality in association with pneumonia during coinfections[14]. It has been noted that home medicines have antibacterial and anti-viral property also that can be proved to strengthen the immune system and fight viruses like coronavirus. Not only home medicine is found to have strong anti-viral properties but also that are potentially active against cytokine storms [15]. A cytokine storm can kill the patients by horribly damaging lungs during the flu virus infection. Usually honey can be used as an effective medicine to treat respiratory ailments, surface infections, diarrhea and several other diseases in Ethiopia and india[16]. Garlic (Allium sativum) seems to be an immune boost and it strengthens the immune system. As it contains allicin it is potentially active against cough and cold [17,18]. Usually Onion found to possess antidiabetic, antihypertensive, antibiotic and antithrombotic properties. Moreover, it exhibits insecticidal and gas odorant properties due to the presence of diethylsulphide and Dimethyl disulphide. Moreover, mixture of honey and ginger exhibited high antimicrobial activity against pathogenic Gram positive and negative bacteria. Moreover honey with lemon juice had high rate of killing on Staphylococcus aureusand Streptococcus pneumoniae[19]. Although, antibiotics such as erythromycin, vancomycin, penicillin, chloramphenicol, clindamycin, trimethoprim, tetracycline, amoxicillin are potentially active against bacteria in terms of causing coinfection associated with SARS and other infectious corono viral diseases, continuous use of antibiotics enabling the organism resistant [20,21]. Usually complications developed in patient due to comorbidities following viral infections. As viral infection associated super bug causing co-infections exacerbates the society by showing antibiotic resistance, there is an urgent need of home medicine as an alternative in order to fending human from the consequences. The present study intended to compare the synergistic effect of honey garlic mixture with onion honey mixture against clinical pathogenic drug resistant bacteria.

Materials And Methods
Study Area, and Period: Present study was conducted at various ISO certified medical labs from September 2020 to March 2021

Sample Collection
Totally 62 samples were collected from patients with cleft palate suspected to corona reported in various medical college hospital prior to antibiotic treatment. Attention paid to collect pus swab intra-orally from the throat and palatal cleft. Samples were collected with the help of trained healthcare provider. Thus samples were transported into Amies transport media (HiMedia, Mumbai, India) and then brought into the microbiology laboratory for further process to be done.

Culturing and Identification
Samples were initially streaked on nutrient agar in order to confirmation of growth and it was subsequently streaked on BHI agar, mannitol salt agar blood agar, (Oxoid) Macconkey agar and SDA by using a inoculation loop separately. Culture plates were then kept in incubator at 37 °C for 24–48 hours. Microorganisms was identified using biochemical kit API Biomeriux,(Biomeriuxpvtltd,Delhi) Sputum staining was done for detecting Mycobacterium tuberculosis.

Antibacterial Susceptibility Testing
In this, Kirby–Bauer disk diffusion method was performed based on the principle set by Clinical Laboratory Standard Institute (CLSI), 2016. Bacterial suspension were prepared and suspended in normal sterile saline. The density of inoculums was determined by evaluation with opacity standard on McFarland 0.5 barium sulphate solution. The test inoculum was evenly spread on the surface of Mueller–Hinton agar (Oxoid). Known concentration of antibiotics disc such as gentamicin ciprofloxacin (5 μg), (10 μg), tetracycline (30 μg), clindamycin (10 μg), chloramphenicol (30 μg), amikacin (30 μg), erythromycin (15 μg), and vancomycin (30 μg) were placed over the medium and then incubated at 37 °C for 16–18 hours. Around the discs, the zone of inhibition was measured, thus obtained results were then classified as sensitive, intermediate, and resistant as per standardized table supplied by CLSI, 2016.

Test Organisms
Isolates with highest drug resistance frequency was further screened. DRSP (Drug resistant S. pneumonia) was screened using pencillin. Methicillin-resistant S. aureus was identified by testing the resistance efficacy of an organism to oxacillin (1 μg) and cefoxitin (30 μg) in disc diffusion method using modified Muller–Hinton agar (Oxoid, Basingstoke, UK). Zone of inhibition was interpreted and grouped into methicillin-sensitive and methicillin-resistant S. aureusin accordance with the CLSI, 2016 guidelines.

Preparation of garlic and onion extracts
Fresh onion and garlic bulbs were procured from Apta market, Tamilnadu, India and cleaned well. Fresh onion and garlic extracts were made using sterilized distilled water followed by their moisture content was reduced by using oven at the temperature ranged 60 °C. Next, the onion and the garlic crimps was grinded well and powdered. 10 gm of each powdered material was transferred into an Erlenmeyer flask containing 100 ml of deionized water and this mixture was allowed to be autoclaved. Then liquid material was filtered aseptically and collected in a sterile flask. Thus prepared extracts with the concentration of 100 μg /ml were properly stored at 40 °C separately for the purpose of experimental study. From this, onion and garlic extracts were diluted with the volume of, 50%, 25%, 12.5% and 6.25% (v/v) for the evaluation of antimicrobial activity.

Antibacterial Susceptibility Testing using garlic and onion
To determine antagonistic activity, Disc diffusion method was done. The paper discs around 6 mm in diameter were prepared by using what man paper and then sterilized using hot air oven at 160 °C for 2 hours. Thus prepared disc was aseptically soaked into the extract. Sterile Mueller Hinten Agar was aseptically poured into sterile culture place and made them solidified. Overnight culture to be tested was inoculated into dried agar plates. Discs impregnated with extracts were aseptically placed on the surface of Agar plates with the help of sterile forceps. Ciprofloxacin disc was used as a control and the plates were incubated at 37 °C for 24 hours. Then zones of inhibitions were examined and the Mean value was calculated from duplicate.
Antibacterial susceptibility testing of honey lemon mixture

In this test, Agar well diffusion was performed. The honey and lemon juice mixture extracts with the equal volume 5+5=10 g, (100 μg/ml) were prepared separately. From this, 50%, 25%, 12.5% and 6.25% (v/v) of extracts of honey lemon mix were prepared by diluting the mixture with appropriate volumes of sterile distilled water for determination of antimicrobial activity. Agar well diffusion technique as described by Aiyelaagbe et al. [22]. Inhibitory zone was measured and the test was continued for three times to obtain mean value.

Evaluation of Minimum Inhibitory Concentration

To determine the Minimum Inhibitory Concentration (MIC), the tube dilution method was performed. Each testing extract, Series of 10 sterile test tubes were kept in the test tube rack and labeled as 1 to 8, remaining three tubes were denoted as broth control tube (BC), growth control tube (GC) and extract control tube (EC), thus 33 tubes were taken and arranged in three racks. In this, three testing extracts namely A(GARLIC) B(ONION) and C(LEMON/HONEY) were analysed. Firstly, 1ml of sterile Mueller Hinton broth was added to all the tubes in three rows, subsequently first tubes of each rows are added with 1ml of testing extract (100%, (100 μg/ml) as mentioned above, thus 1 ml broth and 1ml testing extract were contained in first tube (1:1). Then twofold serial dilution was made by adding 1 ml of mixture into the second tube using another sterile micropipette and vortexes for homogenization. Then mixed thoroughly and serial dilution was continued to till the eighth tubes of each rows reached the dilution of 1 : 128 (0.78 μg/ml) v/v and from the eighth tubes 1 ml was taken and discarded. The GC tube contains bacterial inoculums without extract, BC had only broth and the EC (extract control tube) without bacterial inoculums were maintained. Then 100 μL of testing bacteria was inoculated into each tube except EC (extract control tube). The entire procedures were repeatedly done for three times followed by tubes were incubated at 37 °C for 24 h and the presence and absence of growth were determined by observing turbidity. MIC was noted as the lowest concentration of extract that restrained bacterial growth (no visible growth or turbidity).

Determination of Minimum Bactericidal Concentration

MBC was determined by transferring the nonvisible sign of bacterial growth and turbidity present in MIC tube into sterile nutrient agar plates. In this test, loop full of inoculum was taken from MIC tubes and allowed it into simple streak on these plates. Then plates were incubated at 37 °C for 24 h aerobically. Thus plates showing growth indicated no antibacterial activity; no growth indicated bactericidal and light to moderate growth were recorded as bacteriostatic.

Quality Control

As per the manufacturer's instruction, all the culture media were prepared and the sterility was tested by incubating representative of the batch at 35–37 °C overnight and observing bacterial growth. Those batch of the media showed growth was discarded. To check the quality of the media and antibiotics potentiality, Purchased bacterial strain S. aureus (ATCC-25923) and Streptococcus pneumonia (ATCC63) was used.

Result

Prevalence of cleft palate cases

Table 1 shows the cases with cleft palate reported in hospital visit. A total of 62, male (n=43); female (n=19), highest occurrence of cases reported in group III(defects involving soft palate to alveolus and lip) and this was found to be common.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Defects in soft palate</th>
<th>Defects in hard and soft palate</th>
<th>Defects in soft palate involving lip</th>
<th>Complete bilateral cleft</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>10-20</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 1 Distribution of cleft palate patients associated RTI based on veau classification
Study Population and Patient Characteristics

A total of 62 patients including male (n=43); female (n=19), all the samples exhibited positive growth and yielded 153 isolates, they are Klebsiella pneumonia 9 (5.9%), Staphylococcus aureus 31 (20.26%), E faecalis 7 (3.57%), Pseudomonas aeruginosa 26 (17%), Streptococcus pneumonia 36 (23.52%), Streptococcus pyogenes 25 (16.3%), E coli 11 (7.1%) and Candida albicans 8 (5.2%). Out of 153 isolates, including mono microbial and poly microbial, maximum isolates reported in group iii (soft palate usually involving lip) however, for the purpose of this research, more focus will be on Streptococcus pneumoniae and Streptococcus pyogenes. However no Mycobacterium isolates have been reported out of 62 (n=62) 2 (3.2%) patients have had cardiac problem, 7 (11.29%) showed hypertension 5 were (8.06%) found to have diabetic and 3 (4.9) cases had asthma. The incidence of the coinfection pneumonia was highest among the age group above 60 years elder people.

Prevalence of MRSA

As stated in Figure 1, among isolated S. aureus 31 (20.26%), 5 (16.12%) were screened for methicillin resistance, whereas 21 (83.9) found to be sensitive to methicillin.
Prevalence of DRSP

Among isolated Streptococcus pneumonia 36 (23.52%), 7 (19.4%) were found to be reported with DRSP

Figure 1 Image showing S. aureus on bloodagar

Figure 2 Image showing S. pneumonia on bloodagar

Figure 3 Image showing bacterial growth on bloodagar
Antibiotic Sensitivity Pattern

The isolates with the highest frequency of Streptococcus pneumonia and Staphylococcus aureus were tested against selected 10 antibiotics that were recommended by the Clinical Laboratory Standard. Efficacy of antibiotics against testing isolates found to be varied. More than 50% percent of S. aureus (n = 21) were found to be resistant to vancomycin chloramphenicol and pencillin but least percentage of resistance was observed in erythromycin and ciprofloxacin (9.53%), (Figure 3) however pathogens were sensitive amoxacilin whereas, MRSA (n = 10), Tetracycline with 40% followed by 30% each of penicillin and erythromycin were exhibited resistance. Moreover, all isolates presented high sensitivity to, co-trimoxazole; Amoxacilin, ciprofloxacin, and gentamicin (fig 4). A total of 36 isolates, Streptococcus pneumonia (n = 36) showed obstinate resistance against penicillin, 22% clindamycin 34% and erythromycin 19.4% respectively. Tested isolates had showed high sensitivity to, co-trimoxazole; Amoxacilin, ciprofloxacin, and vancomycin.(fig 3)

Figure 4 Antibiotic Sensitivity Pattern against tested isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>VAN</th>
<th>CLN</th>
<th>ERY</th>
<th>AK</th>
<th>TET</th>
<th>CHL</th>
<th>CPR</th>
<th>COT</th>
<th>PEN</th>
<th>GEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant organism (%)</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

VA = vancomycin; CLN = clindamycin; ERY = erythromycin; Am = Amoxacilin; TET = tetracycline; CHL = chloramphenicol; CPR = ciprofloxacin; COT = co-trimoxazole; GEN = gentamicin
CLN = clindamycin; VAN = vancomycin; ERY = erythromycin; PEN = penicillin; TET = tetracycline; CHL = chloramphenicol; COT = co-trimoxazole; GEN = gentamicin

Disk Diffusion Test

In this study, there are three testing extracts namely A (GARLIC) B (ONION) and C (LEMON/HONEY) were analysed three of labeled extract A to -C, garlic onion extracts and lemon/honey, with the concentration of 100%, 50%, 25%, were tested for their antimicrobial potential on MDR isolates with the highest percentage of S. aureus (isolate no: 7, 9, 17 and 20) methicillin-resistant S. aureus (MRSA isolate no 6) and Streptococcus pneumoniae (isolate no 3, 5, 12, 14 and 26). Thus 10 isolates were subjected into antibiogram study using above extracts. Among all isolates were tested using garlic extracts with the concentration of 100% (100 μg/ml) 50%, 25%, average inhibition zones of (20-22 mm)100 μg/ml, (14±1 mm) 50 μg/ml and (9±1) 25 μg/ml have been noted, whereas effect of onion with the concentration of 100%, 50%, 25%, on all isolates resulted with the inhibition zone 18±1 mm, 11-13 mm and 8 mm respectively. However lemon with honey juice had high rate of killing on tested isolates S. aureus, MRSA and S. pneumoniae. Inhibition zone diameter was ranged from 28±1 mm to 35±1 mm (100μg/ml) 20-22 mm and 14±1 mm.
25μg/ml and compared with 10±1 mm to 30±1 mm for ciprofloxacin at the various concentration used.

**Determination of MIC**

MIC and MBC of the antimicrobial agents were determined with labeled extract A to - C, garlic onion extracts and lemon/honey, with the concentration of 100%, 50%, 25%, on MDR isolates with highest percentage of S. aureus (isolate no: 7, 9, 17 and 20) methicillin-resistant S. aureus (MRSA isolate no 6) and S. pneumoniae (isolate no 3, 5, 12, 14 and 26) among the tested extracts, lemon/honey mixture had potent antibacterial effect turbidity was observed in tubes with the dilution ranged from mean value 12.5% (v/v) (Table 3).

<table>
<thead>
<tr>
<th>Lemon/Honey dilution</th>
<th>Test bacteria</th>
<th>Net (1)</th>
<th>1/2</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
<th>1/32</th>
<th>1/64</th>
<th>1/128</th>
<th>MIC (% v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus -7</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>S. aureus -9</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6.25</td>
<td></td>
</tr>
<tr>
<td>S. aureus -17</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>S. aureus 20</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6.25</td>
<td></td>
</tr>
<tr>
<td>MRSA-6</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>S. pneumoniae 6</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2.50</td>
<td></td>
</tr>
<tr>
<td>S pneumoniae 6</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2.50</td>
<td></td>
</tr>
<tr>
<td>S pneumoniae 6</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>S pneumoniae 6</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>12.5</td>
<td></td>
</tr>
</tbody>
</table>

, no growth (bactericidal); +, growth

**Determination of MBC**

As mentioned above, the most effective lemon honey was further analyzed to determine MBC against 10 isolates. Consequently, the MBC ranged from 6.25 to 2.50 and the results were tabulated (Table 3).

<table>
<thead>
<tr>
<th>Lemon /Honey dilution</th>
<th>Test bacteria</th>
<th>Net (1)</th>
<th>1/2</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
<th>1/32</th>
<th>MBC (% v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus 7</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>2.50</td>
</tr>
<tr>
<td>S. aureus 9</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>12.5</td>
</tr>
</tbody>
</table>
Lemon /Honey dilution

<table>
<thead>
<tr>
<th>Test bacteria</th>
<th>Net 1</th>
<th>1/2</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
<th>1/32</th>
<th>MBC (% v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus 17</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>2.50</td>
</tr>
<tr>
<td>S. aureus 20</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>12.5</td>
</tr>
<tr>
<td>MRSA-6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>12.5</td>
</tr>
<tr>
<td>S. pneumoniae3</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>2.50</td>
</tr>
<tr>
<td>S. pneumoniae5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>12.5</td>
</tr>
<tr>
<td>S. pneumoniae12</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>2.50</td>
</tr>
<tr>
<td>S. pneumoniae14</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>2.50</td>
</tr>
<tr>
<td>S. pneumoniae26</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>12.5</td>
</tr>
</tbody>
</table>

no growth (bactericidal); + , light growth; ++ , moderate growth (bacteriostatic); +++ , heavy growth (no antibacterial potential).

Discussion
This present study describes only about the coinfection caused by bacteria in covid 19 suspected patients with cleft palate problem and the antibacterial potential of home medicine like garlic, onion, lemon and honey on MDR isolates. However study aimed to find the intervention of cleft palate in developing the risk of coronavirus associated bacterial pneumonia.

As the upper respiratory tract is thought to be the site of COVID 19 spread which also hypothetically analysed with the present study results. Viral infection associated super bug causing coinfections exacerbates the society by showing antibiotic resistance, since there is an urgent need of home medicine as an alternative in order to fending human from the consequences. In this regard, present work is intended to analyse antibacterial potential of home medicine like garlic, onion, lemon and honey on MDR isolates with highest percentage of S. aureus (isolate no: 7, 9, 17 and 20), methicillin-resistant S. aureus (MRSA isolate no 6) and Streptococcus pneumonia (isolate no 3, 5, 12, 14 and 26) thus 10 isolates were screened for antibiogram study using above extracts. Isolates were tested using garlic extracts with the concentration of 100% (100 μg/ml), 50%, 25%, average inhibition zones of 20-22 mm (100μg/ml), 14±1mm (50μg/ml) and 9±1 (25μg/ml) were resulted, this obtained results slightly agreed with the study of Hannan et al. [23] whereas the effect of onion with the concentration of 100%, 50%, 25%, against all isolates resulted with the inhibition zone (18±1 mm, 11-13 mm and 8mm) is close proximity to the work of Shinkafi and Dauda[24]. However lemon with honey juice had high rate of killing on tested isolates Staphylococcus aureus, MRSA and Streptococcus pneumoniae with zone of inhibition ranged from 28±1 mm to 35±1 mm (100μg/ml) 20-22 mm and 14±1 mm it is significantly similar to the study of Mshelia et al[25], perhaps it might be due to the highly acidic pH of the honey and lemon juice mixture [26].

According to MIC and MBC values, honey lemon juice mixture exhibited potential bactericidal and bacteriostatic activities against multidrug-resistant tested isolates. This is somewhat concur with the similar study conducted by Mama et al [27]. According to Mirzoeva et al [28], raw honey is found to be effective in making immune system strong since it contains propolis. As citric acid rich in vitamin C, which may potentially active against allergic cough naturally. Honey and lemon juice mixture have better antibacterial activity this may be
given to patient have coinfection associated flu like symptoms causing viruses during acute phase of infection. Moreover it could be hypothetically analysed with COVID19 since it is associated with coinfection pneumonia and sepsis[29] Many COVID-19 patients die of secondary infections rather than the virus itself [30].150 hospitalized COVID-19 patients in Wuhan, China, found that—and half of those who died due to acquired bacterial infections. S. Pneumoniae is thought to be appeared after viral infections, it might be due to the attachment of bacteria to cells that has already been infected by the virus. However the attachment is made by the neuraminidase protein of influenza virus that can cleave the sialic acid of host surface cells in to cryptic acid, this would favour and facilitates the bacterial pneumonia[31].Antibiotic treatment for COVID 19 seems to be a fuel for facilitating the resistance of bacteria [32 33 ]. Adam 2020 [34 ] found that the Traditional Chinese Medicine with the herbal formulations could decrease the period of symptom (average of 2 days), and it helps to reduce body temperature by 1.7 days. Moreover it would help for Shortened length of stay in hospital by 2 days.It provides CT scan improvements 22%, and Clinical cure rate 33 %.It is noteworthy that severe COVID 19 cases might become common cases by 27 percent due to tremendous improvements of immune system marker (lymphocyte) by 70 percent. Many researchers documented that home medicines are helpful to reduce the hospital stay of coronavirus patients by 2 days.As herbs believed to be potential against covid 19 with the above reasons and reducing the complication due to drug resistant bacteria,present study suggests the home medicine lemon honey mixture may reduce the risk of coinfection pneumonia and sepsis in covid 19 suspected cases during acute phase of infection. Moreover we found that the cleft palate anomalies did not show any significant impact on such cases.

**Conclusion**

As herbs believed to be potential against bacterial coinfection in association with covid 19,Present study suggests the home medicine lemon honey mixture may reduce the risk of coinfection pneumonia and sepsis in suspected covid 19 cases during acute phase of infection. Moreover we found that the cleft palate showed no significant impacts in initiating to develop coinfectionincovid 19 suspected cases.

**References**


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