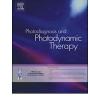


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# Pulsed xenon ultraviolet and non-thermal atmospheric plasma treatments are effective for the disinfection of air in hospital blood sampling rooms

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#### ABSTRACT

*Background and objectives:* Non-thermal atmospheric plasma treatment and pulsed xenon ultraviolet (PX-UV) treatment are widely used in disinfection of hospital environments. However, their effectiveness has not been evaluated against a comparator. The objective of this study is to evaluate their effectiveness in the disinfection of pathogens in the air in hospital blood sampling rooms.

*Methods*: Samples were taken from the air before and after disinfection with PX-UV and non-thermal atmospheric plasma. We counted bacterial colonies and identified the types of bacteria.

*Results:* Non-thermal plasma treatment significantly reduced bacterial counts in the air, the median reduced from 1 before treatment to zero afterwards (p = 0.03). PX-UV treatment also significantly reduced bacterial counts in the air (p = 0.01), the median reduced from 1.5 before treatment to zero afterwards. Pathogens identified in the current study include nosocomial bacteria, such as *Staphylococcus aureus, Staphylococcus epidermidis,* and yeast.

*Conclusion:* Disinfection of blood sampling sites is essential in a health service department. The efficiency of PX-UV and non-thermal atmospheric plasma treatment are comparable in air disinfection.

## 1. Introduction

Hospitals are a source of cross infection [1,2]. This was demonstrated in 2003 by the epidemic of severe acute respiratory syndrome (SARS) in which 128 subjects were infected by a SARS patient who sat in the emergency department of a community hospital awaiting assignment to a hospital bed [1]. A simulated study showed that, due to the length of contact, the cross infection risk between health workers is greater than between patients or between patients and health workers [2]. To make matters worse, a hospital environment is widely contaminated by multidrug resistant organisms [3,4]. Therefore, it is critical to disinfect hospital environments to control cross infection.

The effect of manual cleaning is variable and cleaning is often incomplete even after an intervention to improve cleaning methods [5]. Medical devices used by multiple patients are often inadequately cleaned by manual measures [6]. Therefore automated cleaning systems, such as pulsed xenon ultraviolet (PX-UV), are innovations designed to disinfect hospital environments of different settings, such as wards [7–11], burns units [12], milk feed preparation rooms [13], operation rooms [14,15], surgical sites [16,17], nursing homes [18], long-term acute care facilities [19], isolation rooms [20], and community hospitals [21,22].

Non-thermal atmospheric plasma treatment is also used in prevention of nosocomial infections and other fields, including inactivation of dermatophyte infections in animal models, prevention of bacterial colonization on surgical sutures, and prevention of surgical site infection [23–26], However, the effect is variable [24,27].

Health check-ups, disease prevention, and chronic disease management are an increasingly important subject. The health check-up clinic of the Third XiangYa Hospital sees more than 200 people each

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#### Table 1

Comparison of colony counts before and after plasma treatment and after PX-UV disinfection on air.

| Before plasma treatment on air |                       |                               | After plasma treatment on air |                       |                               |      |
|--------------------------------|-----------------------|-------------------------------|-------------------------------|-----------------------|-------------------------------|------|
| no. samples                    | Median<br>(95%<br>CV) | lowest -<br>highest<br>values | no. samples                   | Median<br>(95%<br>CV) | lowest -<br>highest<br>values | Р    |
| 14                             | 1 (0.89 –<br>1.00)    | 0.00 -<br>6.00                | 14                            | 0 (0.00 -<br>1.00)    | 0.00 -<br>2.00                | 0.03 |
| В                              |                       |                               |                               |                       |                               |      |
| Before PX-UV on air            |                       |                               | After PX-UV on air            |                       |                               |      |
| no. samples                    | Median (95% CV)       | lowest - highest values       | no. samples                   | Median (95% CV)       | lowest - highest values       | Р    |
| 10                             | 1.5(1.00 - 3.5)       | 0.00 - 9.00                   | 10                            | 0 (0.00 - 1.00)       | 0.00 - 2.00                   | 0.01 |

day. Blood sampling, an invasive procedure, is essential for the majority of people attending for evaluation. Disinfection of the blood sampling room is therefore important. However, no comparative study has yet compared the effectiveness of disinfection with PX-UV treatment and non-thermal atmospheric plasma treatment. Here, we compare these two automated methods of disinfecting a blood sampling room.

#### 2. Materials and methods

#### 2.1. Sampling

Sampling sites: This study was conducted in the blood sampling room of the Health Management Center of the Third XiangYa Hospital. The size of the room is 6 m x 6 m. Blood sampling tables are placed in the center of the room. We measured pathogens in the air of the room.

Five plates were used in the analysis of air study. We repeated the PX-UV experiment twice and the non-thermal atmospheric plasma treatment study three times. We did this because a parallel study of the evaluation of the disinfecting effect of PX-UV on a laboratory animal facility showed that PX-UV is effective at sterilizing and the results have been submitted for publication.

Due to errors in sampling, one sample using non-thermal atmospheric plasma treatment was missed. Therefore, the total number of samples analysed was 14 instead of 15.

Sampling time: Samples were taken in the afternoon of the working day at about 2:30 pm before automated disinfection, and then repeated immediately after disinfection.

Sampling methods: For the sampling,  $64 \text{ cm}^2$  tryptic soy agar plates were left open for 30 min. while the control plates were open and covered immediately. Other procedures were the same for both sets of plates.

#### 2.2. Culture count results

The bacterial colonies on each plate were counted and the types of bacteria were classified with matrix-assisted laser desorption/ionization time of flight mass spectrometry (MicroflexLT/SH. BRUKER, Germany). For the surface study, the colony form unit (CFU)/cm<sup>2</sup> is the number of colonies counted on each plate. For the air pathogen culture, results were reported as total CFU per plate. When CFU was over 100 per plate, results were reported as 100.

#### 2.3. Device

The disinfectant efficacy of the PX-UV machine MX-3600 (Xi'An Fukang Air Purification Equipment and Engineering Co. Ltd, Xi'an, China) was initially assessed and we found that it was set at a wavelength of 100 nm–400 nm, a frequency at 3 Hz, and a duration of work

of 6 min, its efficacy was 100% for *Staphylococcus aureus* and *Escherichia coli*. Thus, these parameters were used for this study. The machine was deployed on the floor in the center of the room. Hence, all the air in the room is exposed to UV light.

A non-thermal atmospheric plasma sterilization machine LK/JKF-Y100 (Chengdu Laokan Medical Technology Co., Ltd, Chengdu, China) is routinely used for disinfection each day by the center after blood sampling. During operation, plasma is formed inside the machine and air is sterilized as it passes through the plasma. With air circulating in the room for some time, all of the air in the room should be disinfected. During the study, the machine was placed in the same position as the PX-UV machine of the room, operated as usual, and turned on for 2 h. Samples were collected as in the PX-UV disinfection study.

#### 2.4. Statistical analysis

Data were pooled from the studies and analyzed by performing a Mann-Whitney rank sum test, using MedCalc (version 17) statistical analysis software (MedCalc Software, Ostend, Belgium), to compare the difference between the data before and after disinfection. Threshold statistical significance was set at p < 0.05.

#### 3. Results

#### 3.1. Disinfectant effect of two machines on the air

As shown in Table 1A, non-thermal atmospheric plasma treatment can significantly reduce bacterial colony counts (Mann-Whitney U = 55.50, p = 0.03), the median CFU/cm<sup>2</sup> of 1 before treatment reduced to 0 after treatment. PX-UV reduces bacterial colony counts ((Mann-Whitney U = 17.5, p = 0.01), with the median of CFU/cm<sup>2</sup> being 1.5 before treatment reduced to 0 after treatment (Table 1B).

## 3.2. Pathogens identified in air disinfection study

As shown in Table 2, 30 types of bacteria were identified in the air disinfection study. Of these, 17 pathogens were identified in the non-thermal atmospheric plasma treatment study; 15 were found before treatment and two were found after treatment. In the PX-UV study, 15 pathogens were identified; 11 were identified before treatment and 4 after treatment. Two bacteria (*Micrococcus luteus* and *Staphylococcus hominis*, Table 2) were common to both studies.

#### 4. Discussion

In our study, we found that both PX-UV treatment and non-thermal atmospheric plasma treatment can significantly reduce air pathogen counts and their effects are comparable. Some of the pathogens

#### Table 2

Comparison of the bacteria species using plasma treatment and PX-UV disinfection on air.

| Name of the bacteria identified in the air | Staining | Species identified before plasma treatment | Species identified after plasma treatment | Species identified before<br>PX-UV | Species identified after<br>PX-UV |
|--|----------|--|---|------------------------------------|-----------------------------------|
| Acinetobacter calcoaceticus                | G-       |  |   | +                                  | -                                 |
| Bacillus galactosidilyticus                | G+       | +  | -   |                                    |                                   |
| Bacillus megaterium                        | G+       | +  | -   |                                    |                                   |
| Exiguobacterium aurantiacum                | G+       | +  | -   |                                    |                                   |
| Flavobacterium flevense                    | G-       |  |   | -                                  | +                                 |
| Flavobacterium gelidilacus                 | G-       |  |   | +                                  | _                                 |
| Kocuria marina                             | G+       | +  | -   |                                    |                                   |
| Lactobacillus crispatus                    | G+       |  |   | +                                  | _                                 |
| Lactobacillus fuchuensis                   | G+       |  |   | -                                  | +                                 |
| Lactobacillus ingluviei                    | G+       |  |   | +                                  | _                                 |
| Lactobacillus mali                         | G+       |  |   | +                                  | _                                 |
| Methylobacterium extorquens                | G-       |  |   | +                                  | _                                 |
| Methylobacterium organophilum              | G-       | +  | -   |                                    |                                   |
| Micrococcus luteus                         | G+       | +  | -   | +                                  | _                                 |
| Micrococcus lylae                          | G+       | +  | -   |                                    |                                   |
| Pseudomonas monteilii                      | G-       |  |   | +                                  | _                                 |
| Pseudomonas poae                           | G-       | +  | -   |                                    |                                   |
| Pseudomonas veronii                        | G-       |  |   | +                                  | _                                 |
| Rhizobium radiobacter                      | G-       | +  | -   |                                    |                                   |
| Sphingomonas aerolata                      | G-       |  |   | +                                  | _                                 |
| Staphylococcus arlettae                    | G+       | +  | -   |                                    |                                   |
| Staphylococcus capitis                     | G+       | +  | -   |                                    |                                   |
| Staphylococcus epidermidis                 | G+       | +  | -   |                                    |                                   |
| Staphylococcus epidermidis                 | G+       |  |   | +                                  | +                                 |
| Staphylococcus equorum                     | G+       | +  | _   |                                    |                                   |
| Staphylococcus hominis                     | G+       | +  | -   | -                                  | +                                 |
| Staphylococcus kloosii                     | G+       |  |   | +                                  | -                                 |
| Staphylococcus lugdunensis                 | G+       | _  | +   |                                    |                                   |
| Streptococcus pneumoniae                   | G+       | _  | +   |                                    |                                   |
| Streptococcus pseudopneumoniae             | G+       | +  | _   |                                    |                                   |

G+: Gram Positive, G-: Gram Negative.

+: organism present, -: organism absent.

#### identified in our study commonly cause nosocomial infections.

Nosocomial infection is a worldwide issue that needs to be addressed [1,2]. An investigation in China showed that 1245 patients were diagnosed with a nosocomial infection among 24,390 hospital inpatients [28]. Another study investigated 914 elderly inpatients in a department of gerontology and found that 10% of the patients suffered from nosocomial infection [29]. A study in a hospitalized neonatal intensive care unit found 9% of patients with nosocomial infections [30].

The bacteria identified in this study include those that often cause nosocomial infections, such as *Staphylococcus aureus* [31,32]. Yeasts [32], *Staphylococcus epidermidis* [30,33,34]. *Staphylococcus aureus* and *Staphylococcus epidermidis* are often resistant to multidrug treatment [35,36]. As so few bacteria were identified in each plate we decided against testing them for resistance to antibiotics. Instead we used the published literature as a reference. Therefore, the percentage of drug resistant bacteria in the study is unknown.

Health management and disease prevention play an important role in reducing catastrophic expenditure and the economic burden of society [37]. Cultural and social issues are implicated in the process of chronic disease development, and hence are pivotal to prevention [38]. In China, health examination often involves blood sampling. Taking blood samples is an invasive procedure and is a risk for transmitting nosocomial infections. Indeed, bacteremia is one of the most frequent forms of nosocomial infection [30]. *Staphylococcus aureus* bacteremia is often a serious threat to health [39]. Our study shows that disinfection of the blood sampling environment is important in prevention of nosocomial infections, such as with *S. aureus*.

Non-thermal plasma is a gas that is partially ionized with its energy being stored mostly in free electrons, which can be generated by various electric discharges, such as corona discharges, dielectric barrier discharge, gliding arc, electrospray, plasma jets, and so forth [40,41]. Its biological effects are the results of the effect of heat, ultraviolet radiation, reactive species, and that of charged particles [42].

The types of bacteria identified in the non-thermal atmospheric plasma treatment study in the air were greater than in the study of PX-UV treatment. This is probably caused by two factors. First, plasma treatment was repeated three times, while PX-UV treatment was repeated twice. Second, the total number of people visiting clinic on the days that plasma treatment was used was nearly double than that of the days PX-UV was used. Hence, more types of bacteria were probably brought into the clinic.

In both PX-UV and non-thermal plasma treatment studies, new bacterial species were identified after treatment. These were most likely brought into the room by the automatic ventilation system which was running throughout.

Taken together, our study shows that disinfection of blood sampling sites in a health management department is essential, and PX-UV and non-thermal atmospheric plasma treatment are comparable in killing air-borne pathogens.

#### Author contributions

C.Q.G., Z.H.C., Y.C., and Y.B.L., conceptualized the study and wrote the manuscript. S.N.W., J.J.L., Y.X.L., Z.L., J.J.Q., L.H.C, Y.L., Y.W., and M.M.W conducted the experiments. All authors reviewed the manuscript.

#### **Ethics** approval

Not required.

## **Competing interests**

The authors declare no competing interests.

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