Effect of Clinical X-rays on Methicillin-resistant *Staphylococcus aureus* and Other Bacterial Isolates from Radiology Equipment

K. S. Adebiyi¹, F. O. Ehigiamusoe² and C. E. Oshoma¹*

¹Department of Microbiology, University of Benin, Benin City, Edo State, Nigeria.
²Department of Radiology, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria.

**Authors’ contributions**

This work was carried out in collaboration among all authors. Authors KSA, FOE and CEO designed the study. Authors KSA and CEO performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KSA and CEO managed the analyses of the study. Author FOE managed the literature searches. All authors read and approved the final manuscript.

**Article Information**

DOI: 10.9734/IJPR/2020/v4i130102

**Editor(s):**

(1) Ohanu, Ekechukwu Martin, University of Nigeria, Nsukka, Nigeria.

**Reviewers:**

(1) Syed Umer Jan, University of Balochistan, Pakistan.
(2) Renata Freire Alves Pereira, Federal Fluminense University, Brazil.

Complete Peer review History: [http://www.sdiarticle4.com/review-history/55979](http://www.sdiarticle4.com/review-history/55979)

**Received** 05 February 2020

**Accepted** 10 April 2020

**Published** 17 April 2020

**ABSTRACT**

**Aim:** Methicillin-resistant *Staphylococcus aureus* (MRSA) and other nosocomial pathogens are frequently isolated from the Radiology Department of hospitals. These pathogens are repeatedly exposed to refracted electromagnetic radiations during diagnostic clinical X-ray imaging sessions. This study thus investigated the effect of these refracted radiations on the nosocomial bacteria found within Radiology units and their possible survival mechanism.

**Methodology:** Swab samples were collected from three (3) Radiology units; plain radiography machine, ultrasound machine and mammography machine. The samples were cultured and identified following standard microbiology procedures. The identified bacterial isolates were exposed to X-rays at different Kvp and MAs factors, cell counts and catalase activities of the isolates were determined using standard procedures.

**Results:** The bacterial isolates identified were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Enterococcus faecalis* where *Staphylococcus aureus* had the highest prevalence (29.41%) and *Enterococcus faecalis* (11.76%) was least. The highest survival level to Kvp and MAs exposure was *S. aureus* with values 11.16 ± 0.86 x10³ and 19.50 ± 0.16 x10³.

*Corresponding author: Email: cyprian.oshoma@uniben.edu, oshomacy@yahoo.com;
especially at low doses. In some organisms survive human tolerable clinical x-ray radiations. Resistance and adaptability enable bacteria to tolerate these radiation exposures. Some organisms are continually exposed to varying radiation levels, and their resistance and adaptability enable them to persist in environments where radiation is present.

However, pathogens in these x-ray imaging rooms are not shielded from these radiations. They are continually exposed to varying radiation doses. Surprisingly, some of these pathogens are not killed by these refracted beams of radiation, instead they gradually build up resistance and adapt to the environmental and oxidative stress build up due to these radiation exposures.

Resistance and adaptability enable bacteria to survive human tolerable clinical x-ray radiations especially at low doses. In some organisms, there is a tolerable exposure limit and excessive genotoxic stress such as high-dose radiation, may cause severe DNA lesions and lead to genome instability which leads to mutation. Ionizing radiations generate intracellular radicals (O₂·) which cause oxidative stress, through the increased concentration of active oxygen to a level that exceeds the cell’s defense capacity.

The study revealed that clinical x-ray does not completely eliminate nosocomial pathogens within Radiology Department and MRSA is a formidable bacterium in the units.

## Keywords:
Catalase activity; bacterial survival; kilovoltage peak; milliamperage per seconds; X-ray.

### 1. INTRODUCTION

The Radiology Department of every hospital plays a major role in medical diagnosis, it regularly receives large number of patients resulting in high human traffic which results in the transmission of nosocomial pathogens amongst patients and Radiology Staff. Some of the commonly found pathogens are Staphylococcus aureus, Staphylococcus epidermidis, Candida albicans, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa.

Keywords: Catalase activity; bacterial survival; kilovoltage peak; milliamperage per seconds; X-ray.

Resistance and adaptability enable bacteria to survive human tolerable clinical x-ray radiations especially at low doses. In some organisms however, there is a tolerable exposure limit and excessive genotoxic stress such as high-dose radiation, may cause severe DNA lesions and lead to genome instability which leads to mutation. Ionizing radiations generate intracellular radicals (O₂·) which cause oxidative stress, through the increased concentration of active oxygen to a level that exceeds the cell’s defense capacity. The cellular component target for these highly reactive oxygen species (ROS) are DNA, RNA, proteins and lipids. Much of the damage is caused by hydroxyl radicals generated from hydrogen peroxide molecules (H₂O₂) which cause oxidative stress, through the increased concentration of active oxygen to a level that exceeds the cell’s defense capacity.

Microbial cells have developed enzymatic components including catalase and superoxide dismutase which help to protect the cells’ proteome from oxidative inactivation. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen thereby protecting the cell from oxidative damage due to reactive oxygen species (ROS) which cause oxidative stress, through the increased concentration of active oxygen to a level that exceeds the cell’s defense capacity.

Microbial cells have developed enzymatic components including catalase and superoxide dismutase which help to protect the cells’ proteome from oxidative inactivation. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen thereby protecting the cell from oxidative damage due to reactive oxygen species (ROS) which cause oxidative stress, through the increased concentration of active oxygen to a level that exceeds the cell’s defense capacity.
2.2 Bacterial Enumeration and Characterization

The swab sticks were dipped into 10 ml of sterile distilled water in test tubes and allowed to stand for 30 mins after being slightly whisked. From this stock, a 10-fold dilution was carried out, there after 1.0 ml was transferred using spread plated method, onto plates containing Nutrient Agar and Mannitol Salt Agar respectively. All the plates were incubated at temperature of 37°C for 24h. The method described by Public Health England [17] for estimating bacterial counts was used to enumerate the total viable counts of the isolates. Colonial growths were sub-cultured for purification and purified bacterial isolates were preserved in Nutrient agar (NA) slants. The isolates were characterized by morphology, Gram’s reaction and biochemical test using the scheme in Bergey’s manual of determinative bacteriology [18,19].

2.3 Antibiotic Susceptibility Test

Antibiotic sensitivity of identified bacterial isolates were carried out using Kirby-Bauer disk diffusion susceptibility test with the following antibiotics: septrin (10μg), chloramphenicol (10μg), sparfloxacin (25μg), ciprofloxacin (10μg), amoxillin (30μg), augmentin (25μg), gentamycin (10μg), pefloxacin (10μg), oflaxin (30μg) and streptomycin (25μg). The test organisms were inoculated into sterile nutrient broth in a test tube and incubated at 37°C for 24 h. From the pure culture, 0.1 ml was transferred into solidified Nutrient agar (NA) in a petri-dish and a sterile spreader was used to ensure even distribution on the agar plate. The plates were allowed to air dry for 5 min thereafter the standard antibiotics discs were laid on the inoculated agar. The plates were incubated at 37°C for 24 h. Clear zones around the discs were measured and interpreted as either susceptible, intermediate or resistant. Zones of inhibition ≤14 mm were recorded as Resistant, 15 mm – 17 mm as Intermediate and ≥18 mm as Susceptible [20].

2.4 Determination of Methicillin Resistant Staphylococcus aureus (MRSA)

Sensitivity to Oxacillin was determined using Oxacillin disk placed on a standardized inoculum of S. aureus spread plated on Mannitol salt agar then incubated at 37± 2°C for 24h. Clear zones around the discs were measured and interpreted as either susceptible or resistant for the test organisms to the particular antibiotic. Zones of inhibition ≤14 mm were recorded as Resistant, 15 mm – 17 mm as Intermediate and ≥18 mm as Susceptible [20].

2.5 Detection of mecA Gene from S. aureus Isolates

Strains of S. aureus which showed resistance to Oxacillin were analyzed using molecular techniques to detect the presence of the mecA gene using specific MRSA gene primers. The primer sequences (5’-CCA ATT CCA CAT TGT TTC GGT CAT A- 3’) for mecA gene was used which matches with the sequence of mecA genes published by Zhang et al. [21]. PCR assay was carried out for the detection of mecA (associated with methicillin resistance) using 1X IQ™ Multiplex Power mix (Bio-Rad Laboratories, Hercules, CA, USA) following the manufacturer’s recommendations Electrophoresis of amplicons was performed with 1% agarose gel containing ethidium bromide (EB) 0.5 mg L–1, for 1 h at 100 V in 0.5× TAE buffer (40 mM Tris-HCl, 20 mM Na-acetate, 1 mM EDTA, pH 8.5). A molecular weight marker ladder was included on each gel. Bands were visualized using an UV trans-illuminator [21].

2.6 Exposure of Isolates to X-rays

Pure cultures of the isolates were sub-cultured from a slant into sterile nutrient broth and incubated at 37°C for 24h. A cell suspension was formed using distilled water. An aliquot of 2.0ml was spread plated into petri dishes and incubated at 37°C for 24h. The colonial growth after 24h incubation was counted and recorded as pre-exposure count [22]. At this point, some plates were then kept aside for pre-exposure catalase activity test. The other plates were then exposed to different x-ray radiation factors (at High KVP value of 133.00, Medium KVP value of 86.50, Low KVP value of 40.00 and High MAS value of 180.00, Medium MAs value of 100.00 and Low MAS value of 20.00) [23]. After laboratory exposure, the plates surface was carefully scoped, dissolved in 2.0 ml of distilled water and spread plated into new petri dishes. The plates were incubated at 37°C for 24h. The colonial growth after 24h incubation was counted and recorded as post-exposure count. The mean values from each isolate triplicate plates were calculated and recorded [24]. Likewise, some plates were then kept aside for post-exposure catalase activity test.
2.7 Catalase Activity Test
The plates kept aside for Catalase test were used to study the catalase activities of the isolates before and after laboratory exposure to X-ray. A 0.5 McFarland turbidity standard cell suspension of the isolates was made after carefully scooping the plate contents. The suspensions were transferred into test tubes and 5ml of freshly prepared hydrogen peroxide was introduced into the test tube and incubated in a dark laboratory cupboard for 10mins allowing the isolates to find time to synthesis catalase enzyme to dissociate and dissolve the hydrogen peroxide introduced [25]. To investigate the extent of the isolate’s hydrogen peroxide dissociation ability (catalase test), a known volume (recorded as initial volume) of iron (II) sulphate was titrated from a burette into the presumably remaining (un-dissociated) hydrogen peroxide solution after the cells’ catalyzing activity on it. The titre value of the burette on completion of the left-over (un-dissociated) hydrogen peroxide is used to determine the cell catalase activity of the isolates [26].

2.8 Cellular Catalase Activity of the Isolates
Cellular catalase activity of the isolates was determined by employing Walkley-Black Titration Derivative [27]:

\[
\text{Cat. Activity (umol/min)} = ((\text{Bk-T}) \times M \times 10^6/\text{L} \times I) 
\]

Where:

Bk = Blank (Volume of Hydrogen Peroxide)
T = Titre Value
M = Molarity of Iron (II) sulphate
L = Cell Load (McFarland Standard)
I = Incubation Time
\(10^6\) = Conversion Factor (mol/mins to µmol/min)

The catalase activities of the isolates before and after laboratory exposure to X-ray were calculated and recorded [27].

2.9 Statistical Analysis
Descriptive statistics, analysis of variance and pearson correlation coefficient were used for statistical analysis of obtained data using statistical package for social sciences version 21. Data presentation was done using Microsoft Excel version 2010 [28].

3. RESULTS AND DISCUSSION
Total heterotrophic bacterial count and Staphylococcus aureus counts isolated from the different radiology units are shown in Table 1. The highest number of Staphylococcus aureus (3.61 ± 0.19x10^3 cfu/cm²) and heterotrophic bacterial (8.48 ± 0.2 x10^3 cfu/cm²) were isolated from the Ultrasound unit, followed by Mammogram unit and least was X-ray unit. The high bacterial count in the ultrasound unit is probably due to the absence of ionizing radiation for imaging in ultrasound rooms which might have less harmful effect to the isolates [29].

The identified bacterial isolates and their frequency of occurrence from all the units is shown in Table 2. The highest frequency of occurrence was Staphylococcus aureus while the least was Enterococcus faecalis with values 29.41 and 11.76% respectively. In this study the presence of Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus substilis, Escherichia coli and Enterococcus faecalis were recorded within the sampled radiology units. These microbes are known to be nosocomial due to their colonization of clinical fomites [4,6]. As such, the presence of these microbes might be due to poor sterilization and sanitization procedures carried out on radiology equipment [1]. The resistance to common cleaning agents and antibiotics has made Staphylococcus aureus renown in radiology units [3,4] hence, its high frequency of occurrence than other bacterial isolates.

The antibiotic susceptibility test of the isolates is shown in Table 3. Staphylococcus aureus isolates were highly resistant to most of the antibiotics used including Oxacillin. Other isolates showed a relatively low antibiotics resistance. MRSA is a known multidrug resistant strain of Staphylococcus aureus capable of high-level antibiotic resistance [30] as confirmed in this study using Oxacillin disk test [31] and analysis of data from molecular examination which detected the presence of mecA genes which encodes for resistance to methicillin and other β-lactam antibiotics [30]. All the strains of Staphylococcus aureus from the various units showed the presence of mecA gene as exemplified in Plate 1.
was an increase in cell count difference for KVp isolates before and after laboratory exposure factor. The difference in cell count of the bacterial isolates before and after laboratory exposure to MAs exposure factor is shown in Fig. 2. There was an increase in cell count difference for S. aureus, P. aeruginosa, B. subtilis and E. coli indicating cellular survival. The highest value of 11.16 ± 0.86x10^3 cfu/ml was for S. aureus isolated from X-ray unit followed by S. aureus isolated from mammogram unit (1.50 ± 0.80 x10^3 cfu/ml). The least value-38.50 ± 0.61 x10^3 cfu/ml was from E. faecalis indicating cell death at this exposure factor.

The difference in catalase activity of the bacterial isolates before and after laboratory exposure to KVp exposure factor is shown in Fig. 3. There was an increase in cell catalase activities difference for all S. aureus isolates indicating cellular survival. The highest value of 0.73 ± 0.50 x10^3 umol/min was for S. aureus isolated from X-ray unit followed by S. aureus isolated from

### Table 1. Pre-radiation total heterotrophic bacterial (THBC) and S. aureus counts from the radiology units

<table>
<thead>
<tr>
<th>Isolates</th>
<th>X-ray unit (x10^3 cfu/cm³)</th>
<th>Ultrasound unit (x10^3 cfu/cm³)</th>
<th>Mammogram unit (x10^3 cfu/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THBC</td>
<td>6.28 ± 0.49</td>
<td>8.48 ± 0.32</td>
<td>7.54 ± 0.35</td>
</tr>
<tr>
<td>S. aureus</td>
<td>2.47 ± 0.22</td>
<td>3.61 ± 0.19</td>
<td>1.48 ± 0.17</td>
</tr>
</tbody>
</table>

### Table 2. Frequency of occurrence of bacterial isolates from the radiology units

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>29.41</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>23.53</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>17.65</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>11.76</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>17.65</td>
</tr>
</tbody>
</table>

### Table 3. Antibiotic resistance pattern of bacterial isolates from the radiology units

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th><em>S. aureus</em> (N=5)</th>
<th><em>B. subtilis</em> (N=4)</th>
<th><em>E. coli</em> (N=3)</th>
<th><em>E. faecalis</em> (N=2)</th>
<th><em>P. aeruginosa</em> (N=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%R</td>
<td>%R</td>
<td>%R</td>
<td>%R</td>
<td>%R</td>
</tr>
<tr>
<td>SXT</td>
<td>100</td>
<td>0</td>
<td>50</td>
<td>100</td>
<td>66.9</td>
</tr>
<tr>
<td>CHL</td>
<td>100</td>
<td>50</td>
<td>0</td>
<td>100</td>
<td>33.3</td>
</tr>
<tr>
<td>SPA</td>
<td>100</td>
<td>25</td>
<td>66.7</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>CPX</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AM</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AU</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CN</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>33.3</td>
</tr>
<tr>
<td>PEF</td>
<td>100</td>
<td>25</td>
<td>33.3</td>
<td>0</td>
<td>33.3</td>
</tr>
<tr>
<td>OFX</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>S</td>
<td>100</td>
<td>0</td>
<td>33.3</td>
<td>0</td>
<td>66.9</td>
</tr>
<tr>
<td>OXA</td>
<td>100</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Key:** N = No. of Isolates tested, %R = Percentage Resistance of Isolates, ND = Not Determined, SXT = Septrin, PEF = Pefloxacin, CHL = Chloramphenicol, OFX = Ofloxacin, SPA = Sparfloxacin, S = Streptomycin, CPX = Ciprofloxacin, Am = Amoxicillin, Au = Augmentin, CN = Gentamycin, OXA = Oxacillin
Plate 1. Gel electrophoresis of mecA gene of Staphylococcus aureus from the radiology units
Lane 1: S. aureus isolated from X-ray Unit, Lane 2: S. aureus isolated from Ultrasound Unit. Lane 3: S. aureus isolated from Mammogram Unit, Lane 4: Shows negative control, Lane 5: S. aureus positive control (533 bp), M = DNA Molecular Weight Marker

Fig. 1. Difference in cell count of isolates before and after laboratory exposure to KVp X-ray factor
A= S. aureus (X-ray unit isolate), B = S. aureus (Ultrasound unit isolate), C = S. aureus (Mammogram unit isolate), D = P. aeruginosa, E = B. Subtilis, F = E. coli, G = E. faecalis

Fig. 2. Difference in cell count of isolates after laboratory exposure to MAs X-ray factor
A= S. aureus (X-ray unit isolate), B = S. aureus (Ultrasound unit isolate), C = S. aureus (Mammogram unit isolate), D = P. aeruginosa, E = B. Subtilis, F = E. coli, G = E. faecalis
ultrasound unit (0.22 ± 0.60x10⁶ umol/min). The least value -0.39 ± 0.61x10⁶ umol/min was from E. coli indicating cell death at this exposure factor and E. faecalis recorded no value being catalase negative.

The difference in catalase activities of the bacterial isolates before and after laboratory exposure to MAs exposure factor is shown in Fig. 4. There was an increase in catalase activities difference for S. aureus, P. aeruginosa, B. subtilis and E. coli indicating cellular survival. The highest value of 1.39 ± 0.60x10⁶ umol/min was for S. aureus isolated from X-ray unit followed by S. aureus isolated from mammogram unit (0.61 ± 0.70x10⁶ umol/min). The least value 0.57 ± 0.90x10⁶ umol/min was for E. coli and E. faecalis recorded zero (0) being catalase negative.

The relationship between catalase activity and bacteria survival following exposure to KVP and MAs exposure factors is shown in Fig. 5. The results show that there is a positive correlation between catalase activity and cell survival with R-value of 0.2512 and 0.695 for KVP and MAs exposure factors respectively.

The bacteria X-ray exposure analysis shows cellular survival of isolates after exposure to various X-ray factors. This is because clinical X-rays which are designated human-tolerable are usually technologically re-engineered to attenuate the adverse biological effects of their radiation on living cells [4,33]. Secondly, proaryotes are known to exhibit much higher radiation tolerance than animals and humans [9]. As a result, the genotype profile of the cells may have been preserved using some form of reversion mechanism (such as base and nucleotide excision repair) [10,12] or defense mechanisms (catalase and superoxide dismutase synthesis) [13] thereby protecting the cells.

The Staphylococcus aureus isolate from X-ray unit had the highest survival level to high X-ray exposure factors followed by S. aureus isolate from mammogram unit. This can be attributed to cellular adaptation as both radiology units employ X-ray (ionizing radiation) during imaging unlike ultrasound unit which do not make use of non-ionizing radiation, hence least survival level [34]. Prolong intermittent exposure of the isolate might have resulted to adaptation resulting from the development of adaptive defense mechanism [24] such ability to synthesize catalase enzyme to mitigate the oxidative stress built up by the ionizing radiation [13].

The isolate, Enterococcus faecalis, experienced the most lethal effect for all X-ray exposure factors (KVP and MAs) with a negative value on Y-axis indicating cell death. The inability of the isolate to synthesize and secrete catalase enzyme to resist the oxidative stress induced by X-ray exposures may be responsible for the cell death [13]. Unlike the other isolates which were able to withstand the oxidative stress from the
radiation due to their ability to secrete catalase enzyme.

Statistical analysis shows that there is a positive correlation between catalase activities and cell survival with R value of 0.2512 and 0.6925 for KVP and MAs respectively. This is probable because cellular survival to a large extent was dependent on the ability of the cell to synthesize catalase enzyme which serve as a defense mechanism to the radiation [35]. The difference between the R-value for KVP and MAs exposure factors shows that there is a higher correlation between cell survival and catalase activities of cells exposed to MAs exposure factors hence more cell survival which is probably due to the lower irradiation power of MAs and its extended irradiation time [7] provided for more cellular adaptability to the radiation.

4. CONCLUSION

The application of radiations in clinical X-ray diagnosis promotes the public assumption that X-ray units and equipment may be free from nosocomial microbes. Thereby influencing the level of sanitization practiced by radiographers on X-ray equipment, making them potential fomites for nosocomial pathogen and thus, a
public health threat to patients and Staff. This study reveals the presence of *P. aeruginosa*, *B. Subtilis*, *E. coli*, *E. faecalis* and Methicillin Resistant *Staphylococcus aureus* (MRSA), a public health superbug within the radiology equipment. These isolates expressed cellular adaptiveness when exposed to clinical X-ray irradiation thereby enhancing their ability to survive within the radiology department. This adaptability is aided by the isolates ability to synthesize high level of catalase which helped them to attenuate the harmful effect of the oxidative stress built-up by these clinical radiations.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

**REFERENCES**


© 2020 Adebiyi et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.comreview-history/55979