Comparative Study of Anti Microbial Activity of Dose Dependent Silver Nanoparticle Preparation, Iodophor and Chlorhexidine against Extended Spectrum Beta Lactamase Strains

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Authors’ contributions
This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information
DOI: 10.9734/JPRI/2020/v32i2630837

Editor(s):
(1) Dr. Vasudevan Mani, Qassim University, Saudi Arabia.

Reviewers:
(1) Liany Figuerêdo de Andrade Melo, Universidade Federal de Pernambuco, Brazil.
(2) Suzana Cavalcanti Monteiro de Oliveira, Sao Leopoldo Mandic College, Brazil.
(3) Ferudun Koçer, Kahramanmaras Sulcû Imam University, USKM, Turkey.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59827

Received 06 July 2020
Accepted 12 September 2020
Published 04 November 2020

ABSTRACT
Iodophor, chlorhexidine and silver nanoparticle preparation were tested against Extended spectrum beta lactamase strains (ESBL strains) in this study. This was done in order to compare the antibacterial activities of 5% iodophor, 4% chlorhexidine and silver nanoparticle preparation. A major problem for chemotherapy of bacterial agents in today’s world is the development of multi drug resistance. ESBL is one of the most common problematic multidrug resistant pathogens. Beta lactamase are enzymes which are produced by certain kinds of bacteria. They have the ability to breakdown the active ingredient in certain common antibiotics and thus making them ineffective. Iodophor and chlorhexidine are chemical disinfectants which show antimicrobial activity. Iodine is said to be one of the most lethal agents that enters the cell and inhibits synthesis of proteins. Iodophors are less toxic and they do not irritate the skin when used for disinfection. Chlorhexidine

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is also a commonly used antiseptic and they are very commonly used as a disinfectant before surgical procedures and for sterilisation procedures. Silver nanoparticles, another component tested against ESBL strain in this study is a biological preparation. Many studies have described the antimicrobial properties of silver and it is proven to be a powerful bactericidal agent as it can effectively cross biological membranes. Although considered to be toxic at high concentrations in human beings, less than 1% concentration of silver nanoparticle preparation is used for controlling bacteria in dental therapies, wounds, burns and catheters.

Keywords: Anti microbial; ESBL; iodophor; chlorhexidine; silver nanoparticle; drug resistance.

1. INTRODUCTION

ESBL strains (Extended spectrum beta lactamase strains) are enzymes which are produced by certain kinds of bacteria. They have the ability to breakdown the active ingredient in certain common antibiotics and thus making them ineffective. They are commonly associated with wound infection, surgical or accident injury. They show multiple drug resistance, survive in the atmosphere for long and can act as a source of infection for other patients [1]. Since it’s prevalent in the hospital environment, eliminating them from surgical sites is very important in order to prevent postoperative complications. There are different tests that help confirm the presence of ESBL strains. Those include double-disk synergy test, three-dimensional test, inhibitor-potentiated disk-diffusion test, disk approximation test. A major problem for chemotherapy of bacterial agents in today’s world is the development of multi drug resistance [2]. ESBL is one of the most common problematic multidrug resistant pathogens [3,4]. Two chemicals are used as disinfectants on skin, namely, iodophor and 4% chlorhexidine [5]. Many strains are resistant to chemical disinfectant also. In this study iodophor, chlorhexidine and silver nanoparticle preparation were tested against ESBL strains.

Silver nanoparticles are prepared biologically as other routes of nanoparticle preparation are toxic to the environment [6]. Use of nanoparticles of certain size, shape and morphology is also economical and they have great potential and stability [7]. Silver nanoparticles are mainly known to exhibit properties like stability and catalytic activities, thus making them a preferred material in the field of medicine [8,9,10]. Nanotechnology and nanomedicine contribute immensely in the development of nanomaterials with the ability to interact with biological forms or entities. Among all the nanotechnological wonders, metal nanoparticles, as used in this study have attracted major scientific interests because of the bactericidal properties shown by metals like silver. Many studies have described the antimicrobial properties of silver and its proven to be a powerful bactericidal agent as it can effectively cross biological membranes [11]. Silver nanoparticles have however also proven to be harmful to human cells at high concentrations and hence it is essential to elaborate materials with low concentration of silver nanoparticles in order to have a bactericidal effect without being cytotoxic [12,13].

Chlorhexidine is an antimicrobial agent that acts in a broad spectrum causing damage to the cell membrane of microorganisms at higher concentrations [14]. It is an effective mouth cleanser and has a variety of uses in the field of dentistry and health due to its disinfectant nature [15]. It has significant effect on gram positive and gram negative bacteria, various fungi and viruses [16]. Chlorhexidine is used as an antiseptic for disinfection of skin before surgeries and also to sterilise surgical instruments [17].

Iodophor (polyvinylypyrrolidone iodine) is a preparation containing iodine. Iodine is said to be one of the most lethal agents that enters the cell and inhibit synthesis of proteins. It is also used as a disinfectant and an antiseptic for wounds and bruises [18]. It is also used as a sanitizer in the brewing and dairy industries. Certain studies have proven that even iodophor has a significant effect on both gram positive and gram negative bacteria, fungi and viruses [19]. However, some other studies have proven that the effect against biofilms is lesser than other disinfectants [20]. Iodophors are said to be less toxic than most disinfectants, and do not cause any irritation to the skin, although they leave a yellowish residue on surfaces.

The diverse mechanism of anti bacterial activity of silver nanoparticle preparation, iodophor and chlorhexidine against ESBL strains was explored in this study.
2. MATERIALS AND METHODS

The test used in this study is called a disk diffusion test or agar diffusion test or Kirby-Bauer test. The main materials used are 4% Chlorhexidine, 5% iodine, silver nanoparticle preparation and high media sterile discs (code number: DD036). The test is done against Pseudomonas ESBL strains (standard suspension). It could be confirmed that they were ESBL producing strains as they were resistant to cefotaxime and showed susceptibility to cefotaxime clavulanic acid. 5 sterile paper discs were first soaked in 5% iodine for 10 minutes and transferred to a Petri dish and dried in a hot air oven at 50°C for 30 minutes. Then 3 sterile paper discs were taken with dose dependent silver nanoparticle preparation of 50 µl, 100 µl and 150 µl each and transferred to another Petri dish. They were also placed in the hot air oven at 50°C. These dry discs were placed on the surface of Muller Hinton agar coated with ESBL (overnight culture made suspension). The plates were incubated for 24 hours aerobically at 37°C. After the incubation the plates were checked for a zone of inhibition. Three other sterile discs were taken and dipped in chlorhexidine then transferred to a Petri dish. They were then placed in the hot air over at 50°C. These discs were also later incubated and checked for results.

3. RESULTS AND DISCUSSION

Iodophor, chlorhexidine and silver nanoparticle preparation were tested against ESBL strains and the results were obtained. Chlorhexidine, iodophor preparation and silver nanoparticle preparation are all proved to be excellent antimicrobial agents [21]. However that property differs in this particular strain of ESBL. According to the results of this study both iodophor and chlorhexidine failed to show antimicrobial activity against ESBL strains whereas silver nanoparticle preparation was very effective. The zone of inhibition was measured for each dose dependent disc (The doses were 50 µl, 100 µl and 150 µl each) and found out to be 1.8 cm , 2 cm and 2.1 cm respectively for each of the discs (Table 1). Thus, the zone of inhibition measured showed the susceptibility of the bacteria in the measured circumference.

ESBL strains are resistant to most antibiotics like penicillin, cephalosporins etc [22,23]. Therefore finding a preparation against its activity is necessary as far as its prevalence is concerned.

Fig. 1. Anti microbial activity of silver nanoparticles against Pseudomonas ESBL is effective as the zone of inhibition is maximum

Table 1. Zone of inhibition showing the effective anti microbial activity of different dosed silver nanoparticle preparation against Pseudomonas ESBL strains

<table>
<thead>
<tr>
<th>Doses of discs</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>50µl</td>
<td>18 mm</td>
</tr>
<tr>
<td>100µl</td>
<td>20 mm</td>
</tr>
<tr>
<td>150µl</td>
<td>21 mm</td>
</tr>
</tbody>
</table>

Table 2. Table comparing the zone of inhibition of chlorhexidine, Iodine and Silver nanoparticle preparation

<table>
<thead>
<tr>
<th></th>
<th>Chlorhexidine (4%)</th>
<th>Iodine (5%)</th>
<th>Silver nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 µl</td>
<td>100 µl</td>
<td>150 µl</td>
</tr>
<tr>
<td>Mean zone of inhibition</td>
<td>0</td>
<td>0</td>
<td>18 mm, 20 mm, 21 mm</td>
</tr>
</tbody>
</table>
Fig. 2. Bar chart comparing the zone of inhibition depending on dose of silver nanoparticles

4. CONCLUSION

ESBL strains generally have multiple drug resistance. In many patients the systemic and the topical antiseptics are not effective. This study proves the resistance of Pseudomonas ESBL strains against topical preparations of iodophor and chlorhexidine. Even though chlorhexidine and iodophor seemed to show good antimicrobial activity generally, it differs for this particular strain of ESBL. For such strains topical preparation with silver nanoparticles will be beneficial in clearing the surface lesions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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