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# Effectiveness of amikacin administered by autoinjector compared to manual injection on infected excision wound model of Wistar rats

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ABSTRACT

To evaluate the effectiveness of amikacin administered by autoinjector compared to manual injection on infected excision wound model of Wistar rats. Randomly bred 14 Wistar rats of either sex weighing 180 to 230 g were used for the present study. The study has the approval of the Institutional Animal Ethical Committee. *Pseudomonas aeruginosa* was used for infecting the wounds. 1 mL of blood was withdrawn aseptically from the orbital sinus under isoflurane anaesthesia, and the biochemical parameters were carried out. All results were expressed as mean ± SEM, and the results were compared statistically by one-way ANOVA using Sigma Plot 13. P-value < 0.05 was considered statistically significant. The biochemical parameters in the study was more or less similar. The infected rats treated with amikacin showed faster wound contraction compared to control. This study concludes the effectiveness of amikacin administered through autoinjectors and manual injection in infected excision wound model as similar. Therefore, amikacin autoinjector is a better choice to manual injection to overcome from wound infections if it is administered at the right time in case of emergency or whenever required. Injury and wound infection are common in natural and manmade disasters. Serious bacterial wound infections are a potential threat to open injuries. As accessibility to the primary health centre or hospital may not be easy or possible during disasters and to overcome such a situation, an antibacterial autoinjector would be useful.

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## INTRODUCTION

Injury and wound infection are common in natural and manmade disasters. Serious bacterial wound infections are a potential threat to open injuries. These bacterial infections may cause tetanus or gas gangrene which in turn may end up in long term disabilities like a chronic wound, bone infection and even death. These infections are of great concern when injured persons present late for definitive care. In disasters, the injured survivors may exceed available trauma care capacity. Timely and appropriate intervention with an antibiotic is mandatory to avoid complications and further morbidity and mortality. As accessibility to the primary health centre or hospital may not be easy or possible during

disasters and thus getting appropriate expert care and antibiotic support will be a question. So, to overcome such a situation, an antibacterial autoinjector would be useful (Vijayaraghavan, 2020).

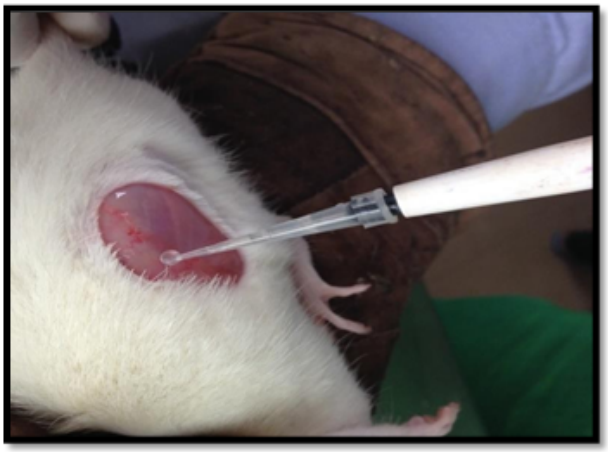

**Figure 1: Representative photo of excision wound in rat inoculated with *Pseudomonas aeruginosa***

**Figure 2: Rats with wound Infection in metabolic cage**

The wound is a loss of cellular and functional continuity of living tissues. Wound healing has got different phases in a sequence – haemostasis, inflammation, tissue formation, and tissue remodelling (Öztürk and Ermertcan, 2011; Eming *et al.*, 2014). Haemostasis is characterised by vasoconstriction and blood clotting. This prevents blood loss and provides a provisional matrix for cell migration. There will be some complex interaction of cells in the epidermis, dermis and simultaneous release of mediators from inflammatory cells, fibroblast and keratinocytes. There will be the formation of growth factors by local and migratory cells which stimulate migration of fibroblasts into the wounded area, which proliferate to form an extracellular matrix. The migration and proliferation of keratinocytes continue until the wound get entirely covered. Another vital process involved is angiogenesis which involves migration, proliferation and organisation of vascular endothelial cells. The matrix formation and epithelialisation depend on angiogenesis. Complete healing occurs only after knitting the wounded surface firmly by collagen (Kumar *et al.*, 2007). The wound can occur due to severe burns, tissue damage and trauma or in diseases like diabetes. Whatever be the reason behind the wound, complete and rapid healing is critical during medical interventions.

Wound infections are widespread in developing countries than in developed countries (Sasidharan *et al.*, 2010). Infection can slow down wound healing, even though it is a natural process (Subramoniam *et al.*, 2001). The clinical signs and symptoms of infection include pain, erythema, oedema, heat, purulent exudates, serous exudate with concurrent inflammation, delayed healing, discolouration of granulation tissue, friable granulation tissue, pocketing at the base, foul odour and wound breakdown (Gardner *et al.*, 2001). *Pseudomonas* and *Staphylococcus* are major organisms often isolated from infected wounds. They are capable of forming a biofilm in the wound surface, and they produce an enzyme – protease responsible for the degradation of the extracellular matrix essential for wound healing.

Amikacin is an aminoglycoside antibiotic highly effective against gram-negative organism like *Pseudomonas*. It inhibits bacterial protein synthesis and produces a bactericidal effect. Hence, the bactericidal effect of the drug can reduce the burden of microorganism and reduction in biofilm formation and thereby can hasten wound healing. This study was carried out to show the effectiveness of amikacin autoinjector compared with manual injection in wound infection.

## MATERIALS AND METHODS

### Animals

Randomly bred 14 Wistar rats of either sex weighing 180 to 230 g were used for the present study. The animals were housed and maintained in polypropylene cages in Centre for Laboratory and Animal Research (CLAR), Saveetha University as per the guidelines of Committee for Control and Supervision of Experiments on Animals (CPCSEA). They were fed with drinking water and commercially available pellets (VRK Nutritional Solutions, Chennai India) ad libitum. The study has the approval of the Institutional Animal Ethical Committee.

**Table 1: Biochemical parameters estimated in control group and infected wound models treated with amikacin autoinjector and manual injection groups.**

| S.No | Parameter | Control | Autoinjector | Manual | Statistical Analysis |
|---|---|---|---|---|---|
| 1 | Total Protein (g/dL) | 6.75 ±0.10 | 7.13 ± 0.38 | 6.45 ± 0.14 | F 1.389<br>P 0.290 |
| 2 | Albumin (g/dL) | 3 .98 ± 0.33 | 3.45 ± 0.24 | 3.60 ± 0.29 | F 0.942<br>P 0.419 |
| 3 | Globulin ( g/dL) | 2.78 ± 0.24 | 3.68 ± 0.41 | 3.24 ± 0.28 | F 1.686<br>P 0.230 |
| 4 | A/G ratio | 1.50 ± 0.28 | 1.02 ± 0.17 | 1.13 ± 0.10 | F 1.603<br>P 0.245 |
| 5 | C-Reactive Protein | 0.67 ± 0.11 | 0.76 ± 0.08 | 0.79 ± 0.125 | F 0.322<br>P 0.731 |

Values aremean ± SE (n= 4 in control andmanual injection group and n=6 in autoinjector group)

**Table 2: Percentage of wound contraction in control group and infected wound models treated with amikacin autoinjector and manual injection groups.**

| S. No | Param-eter | Control (Wound contraction %) | Autoinjector (Wound contraction %) | Manual (Wound contraction %) | Statistical Analysis |
|---|---|---|---|---|---|
| 1 | 4th day | 0.64 ± 0.14 | 0.83 ± 0.04 | 0.84 ± 0.03 | P = 0.153 |
| 2 | 8th day | 10.86 ± 0.09 | 65.60 ± 0.73* | 63.93 ± 0.72 | P < 0.001 |
| 3 | 12th day | 21.10 ± 0.41 | 83.64 ± 0.44* | 80.61 ± 0.29 | P < 0.001 |
| 4 | 16th day | 32.88 ± 0.11 | 92.67± 0.45* | 91.13 ± 0.36 | P < 0.001 |

Statistical Analysis was done with One Way ANOV A with Dunnett's Test P < 0.05 was considered statistically significant where n =4 in control and manual injection and n = 6 in autoinjector . Values are mean SE* indicates statistical significance compared to control.

### Bacteria for wound infection

*Pseudomonas aeruginosa* was obtained from the Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (Chennai, India) for the study. The organism was maintained in nutrient agar slant (Hi-Media Pvt. Ltd., India) at 4°C.

### Excision wound model and infection

The animals were randomly allocated into three groups. Group1 served as a control group with four animals. Group 2, with six animals on amikacin autoinjector and group 3 with four animals on amikacin manual injection. Since amikacin autoinjector is a new device, more animals were used for the wound infection study. Excision wound model was used for this study. A pre-decided area in the dorsal surface of the rats was shaved, and wounds measuring around 500 $mm^2$ (Murthy *et al.*, 2013) were created using a sterile surgical blade under isofluorane anaesthesia aseptically. The wound was infected by instilling 100 $\mu$l of *Pseudomonas aeruginosa* using a micropipette in a biosafety cabinet(Type 2) and the animals were observed for 4 hours (Figure 1). The rats were then housed in separate metabolic cages to develop infection (Figure 2).

### Drug administration

The infected animals in Group 1 were kept as control. Group 2 and group 3 were administered with 63 mg/mL of amikacin i.p from the fourth day, by autoinjector and manual injection (1.2 mL) continuously for four days.

### Rate of wound contraction

Wound size was measured every four days interval ($4^{th}$, $8^{th}$, $12^{th}$ and $16^{th}$ day) with the help of butter paper and graph paper and the percentage of wound the contraction was calculated using the following formula (Figure 3). The percentage of wound contraction was calculated by dividing the difference in

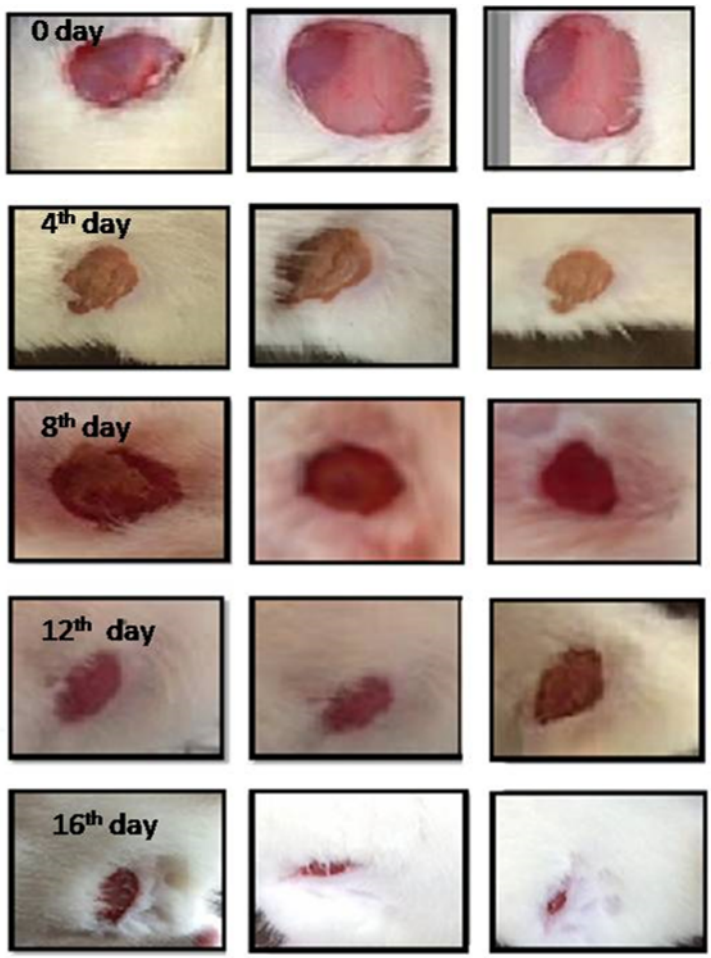


**Figure 3: Representative photo of wound contraction in rat on day 0,4$^{th}$ ,8$^{th}$ 12$^{th}$ and 16 $^{th}$ day of wound infection of control, autoinjector and Manual injection (left to right)**

wound area of a particular day from the zero-day by zero-day area and then multiplying it with hundred (Sadaf *et al.*, 2006).

### Blood sample collection

On the 8$^{th}$ day, 1 mL of blood was withdrawn aseptically from the orbital sinus under isoflurane anaesthesia, and the biochemical parameters were carried out. After the withdrawal of blood, the animals were returned to the metabolic cages and observed for wound contraction.

### The blood biochemical parameters

Total protein, albumin, globulin and C-reactive protein (CRP) were estimated using autoanalyser. The turbidometric method was used for C-reactive protein.

### Statistical analysis

All results were expressed as mean ± SEM, and the results were compared statistically by one-way ANOVA using Sigma Plot 13. P-value < 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

Biochemical parameters like total protein, albumin, globulin and C-reactive protein was analysed. Total protein was 6.75 ± 0.10 g/dL for control. Though there was little increase in the autoinjector group, 7.13 ± 0.38 g/dL it was not statistically significant. In manual injection, the total protein was 6.45 ± 0.14 g/dL. The albumin level were 3 .98 ± 0.33 g/dL, 3.45 ± 0.24 g/dL and 3.60 ± 0.29 g/dL in the control, autoinjector and manual group respectively. The globulin also was similarly with a slight increase in the autoinjector group but not clinically significant. The globulin level was 2.78 ± 0.24 g/dL, 3.68 ± 0.41g/dL and 3.23 ± 0.28 g/dL; the albumin- globulin ratio was 1.50 ± 0.28, 1.023 ± 0.17 and 1.13 ± 0.10 for the control, autoinjector group and manual respectively. The C-reactive protein was 0.67 ± 0.11 mg/dL for control group and for autoinjector and manual injection group, it was 0.76 ± 0.08 mg/dL and 0.79 ± 0.13 mg/dL respectively (Table 1).

The wound in all three groups was almost the same in the beginning. The wound closure on the fourth

day was 0.64 ± 0.14 %, 0.83 ± 0.04% and 0.84 ± 0.03% respectively, for the control, autoinjectors and manual injection group. There was a significant increase in healing ($P < 0.001$) on day eight and the wound closure was 10.86 ± 0.09 %, 65.60 ± 0.73 % and 63.93 ± 0.72 % for control, autoinjector and manual injection, respectively. On day 12 still better-wound closure was observed with 21.100 ± 0.41 %, 83.64 ± 0.44 % and 80.61 ± 0.29% for control, autoinjectors and manual injection, respectively ($P < 0.001$). On day 16 the wound closure was 32.88 ± 0.11 %, 92.67 ± 0.45 % and 91.13 ± 0.36 % for control, autoinjectors and manual respectively with a high significance from control (0.001) (Table 2 and Figure 3).

Rat models are excellent for skin wound healing study as it can be used in terms of size, shape, type and depth of wound injury. They are selected for skin wound healing model because of their small size, ready availability and economical with limited housing facility. Both incision and excision wound models can be prepared to study the healing effect on the dorsum of the animal because they will not allow the animal to reach and manipulate the wound. The skin of rat and humans have a more or less similar pattern of the epidermis, basement membrane and hair follicle and dermis. In this study, the excision wound model was used.

During any injury, the body responds by restoring tissue injury by the synthesis of a connective tissue matrix at the site of the wound. The wound is strengthened by the fibrous protein, collagen, which is the major component of the extracellular matrix. The increase in hydroxyl proline content supports the increased migration of fibroblast cells, epithelial cells and collagen for the synthesis of the extracellular matrix. A decrease in hydroxyl proline can prolong the inflammatory phase of wound healing and then inhibits both epithelial regeneration and proliferation of fibroblast. Wound contraction is a necessary feature for the healing process (Arul *et al.*, 2007).

Contaminated wounds, penetrating wounds, abdominal trauma, compound fractures, lacerations greater than 5 cm, wounds with devitalised tissue, high-risk anatomical sites like hand or foot are at high risk to become infected. Antibiotic prophylaxis is indicated in Injuries requiring surgical intervention within 2 hr. Injury and wound infections are quite common in natural and manmade disasters. Hence, in this study, wound infection with *Pseudomonas* was created to check the effectiveness of amikacin autoinjector in comparison to manual injection as it is one of the organisms often isolated from infected wounds. They produce an enzyme – *protease* responsible for the degradation of the extracellular matrix essential for wound healing and thus can delay wound healing. Amikacin is effective against gram-negative organism like *Pseudomonas*. The bactericidal effect of amikacin might have reduced the burden of microorganism and reduction in biofilm formation and thereby could hasten wound healing. On the other hand, the delayed wound healing in the infected control group may be due to the presence of microorganism and their metabolites (Sasidharan *et al.*, 2010).

The wound closure was slow in the beginning, and once the animal showed a response to the antibacterial agent, the closure rate was faster in the treated group compared to control. The wound closure was significant from day eight, and the wound closure rate was faster in the treated group compared to control. More than 90% closure of the wound was found on day 16 in amikacin treated groups. Control group took more than 22 days to get completely closed. The antibacterial activity of the antibiotic could substantially reduce the burden of the wound pathogen leading to complete and proper wound healing.

The animal weight, food and water intake were monitored regularly. The wound in the untreated group was wet and with malodour due to infection. There could be a link between malodour and delayed wound healing. Malodour was reduced in the treated group, which may be due to a reduction in microbial presence due to antibiotic therapy and the restart of healing (Kalinski *et al.*, 2005; Jørgensen *et al.*, 2005).

Albumin is a gross indicator of nutritional and fluid status. It is needed for tissue synthesis and fighting against infection. It serves as building blocks of all cells and body tissues along with globulin and total protein. Globulin was slightly increased in the autoinjector group but was not significantly high to indicate the superiority of the treatment. Systemic infection may have a more profound effect. Since the wound is topical, changes could not be detected.

C- reactive protein (CRP) is commonly used as a diagnostic measure to identify wounds which require antimicrobial therapy (Kingsley and Jones, 2008). It is a marker for acute inflammation. It is reasonable to presume that CRP levels may get elevated because of infection and fall in response to effective antimicrobial therapy. Many acute inflammatory conditions are diagnosed with its estimation alone or with ESR, WBC count (Michail *et al.*, 2013). In response to acute and chronic inflammatory events, it will be secreted from macrophages,

adipocytes and hepatocytes. Persistently elevated CRP is seen in non-healing chronic wounds (Wright and Khan, 2010). Within 24-48 hrs, the changes in CRP levels can occur in response to the presence or withdrawal of the stimulation, such as a bacterial infection. The biochemical parameters were not significant from control as the infection was not a systemic one.The effect of amikacin autoinjector alone (Anitha *et al.*, 2016) and in combination with cefazolin (Geetha *et al.*, 2016) was tested for biochemical and oxidative stress parameters and found to be similar with manual injection.

## CONCLUSIONS

The infected rats treated with amikacin showed faster wound contraction compared to control. This study concludes the effectiveness of amikacin administered through autoinjectors and manual injection in infected excision wound model as similar. Therefore, amikacin autoinjector is a better choice to manual injection to overcome from wound infections if it is administered at the right time in case of emergency or whenever required.

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### Conflict of interest

The authors declare that they have no conflict of interest for this study.

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