Research Article

The Novel Role of Healing from Bacterial Infections of Lower Limb Open Fractures by X-Ray Exposure

Ali A. Mahdi, Tuqa S. Al-Salmani, and Mustafa M. Al-Qaisi

College of Health & Medical Technology, Middle Technical University, Baghdad, Iraq

Correspondence should be addressed to Tuqa S. Al-Salmani; tuqa.sami86@gmail.com

Received 28 August 2019; Revised 25 January 2020; Accepted 21 February 2020; Published 19 March 2020

Academic Editor: Barbara H. Iglewski

Copyright © 2020 Ali A. Mahdi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

An open fracture refers to a break in the skin, which is exposed to microbial contamination and eventually leads to most complicated infections. X-rays can kill bacteria by causing irreversible DNA damage. Objective. To confirm the role of X-ray exposure in treating infected wound fractures at the lower limb and determine X-ray exposure times.

Methods. Fifty-one wound swabs were collected from patients with infected open fractures at the lower limb with grade II, IIIA, B, and C according to the Gustilo and Anderson classification system and then cultured. The bacterial isolates were identified by biochemical tests and the VITEK-2 System and tested against several antibiotics. The X-ray exposure was done for open fractures by radiography (at kV 133 and 5 milliamperes).

Results. The higher isolation rate was recorded for Staphylococcus aureus with 21 (41.2%) isolates, and most of them (20, 95.2%) were isolated from grade II fractures. The isolation rate of Gram-negative bacteria was 25.5% for Escherichia coli with 13 isolates, 19.6% for Pseudomonas aeruginosa with 10 isolates, and 13.7% for Klebsiella pneumoniae with 7 isolates, most of which were isolated from grade III fractures. The isolation rate of P. aeruginosa was 60% (6 isolates) from grade IIIA and 71.4% (5 isolates) from grade IIIB fractures for K. pneumoniae, while for E. coli it was 69.2% (9 isolates) from grade IIIC. All the bacterial isolates recorded high levels of antibiotic resistance against most tested antibiotics. Wound cultures of grade II fractures appeared sterile after the first X-ray exposure, and these woundswere infected with S. aureus or P. aeruginosa. However, cultures of grade IIIA and IIIB fractures appeared sterile after the second X-ray exposure for all isolated bacteria, except for S. aureus (grade IIIA fractures) after the third X-ray exposure. Grade IIIC fractures showed sterile culture after the third X-ray exposure for wounds infected with P. aeruginosa and E. coli. Conclusions. The study concluded that X-ray exposure showed high effectiveness in treating infected open fractures.

Infections remain the most complicated problem among these wounds [4]. Yearly, the incidence of open fractures recorded as 11.5 per 100 000 persons and >70% involve the lower limb [5]. The severity of open fractures was classified according to the Gustilo and Anderson classification as grades I, II, and III, and grade III was subclassified into IIIA, IIIB, and IIIC depending on the extent of contamination and the degree of soft tissue damage [6]. Hazard infection is 0%–7% for grade I, 0%–11% for grade II, 2%–36% for grade III, and up to 44% for the grade IIIC subtype [7, 8]. The problem that accompanies open fractures is the infection; therefore, an important goal in open fractures treatment is preventing infection [9]. The first application of antibiotics in the treatment of open fractures started during World War II by using penicillin [10]. Antibiotics are now routinely
administered as part of the trauma management protocol for open fractures, leading to an increase in the antimicrobial resistance of open wound fractures [11, 12]. Eventually, treating open fractures became very difficult, and choosing another role in treating became necessary.

X-rays are one type of ionizing radiation that has a penetrating ability to most tissues and kill bacteria by causing irreversible damage to DNA. This radiation can effectively kill many types of Gram-negative bacteria such as E. coli, P. aeruginosa, and Salmonella species [13–15]. The present study aims at proving the role of X-ray exposure in the healing of infected open fractures and determining the optimum number of X-ray exposure for healing from different bacterial causative agents.

2. Materials and Methods

2.1. Study Design. The study design of the present study is the experimental research design. The sample size of the study was not calculated due to the limitation of the research duration. The figures throughout the study were drawn using Microsoft Excel 2010. The outcomes of the study were analyzed statistically using SPSS (version 23) by using Fisher’s exact test.

2.2. Sample Collection. Fifty-one wound swabs were collected from patients with severe inflamed open fractures following fixation management. These wound swabs were cultured on blood agar and MacConkey agar media. The severity of these wound fractures was classified into grade II and grade IIIA, B, and C according to the Gustilo and Anderson classification [6]. They were located at the lower limb of patients admitted (Dowaly Private Hospital) during the period from February 2017 to September 2018.

2.3. Bacterial Identification. All collected wound swabs were cultured on blood agar and MacConkey agar for screening. Whole bacterial isolates were identified to species level by the different standard microbiological and biochemical tests [16], and VITEK-2 System was used to confirm the identification.

2.4. Antibiotic Susceptibility Test. The disk diffusion method was utilized to detect the susceptibility pattern of bacterial isolates against antibiotics. The tested antibiotics can be classified into several classes as follows: (I) aminoglycosides: amikacin (AK) 30 µg and gentamicin (CN) 10 µg, (II) penicillins: ampicillin (AM) 10 µg and oxacillin (OX) 5 µg, (III) penicillin combinations: amoxicillin/clavulanic acid (AMC) 20/10 µg, (IV) cephalosporins: second-generation drugs including cefoxitin (FOX) 30 µg, third-generation drugs including ceftriaxone (CTR) 30 µg and ceftazidime (CAZ) 30 µg, and fourth-generation drugs including cefepime (CPM) 30 µg, (V) carbapenems: imipenem (IPM) 10 µg and ertapenem (ETP) 10 µg, (VI) fluoroquinolones: ciprofloxacin (CIP) 5 µg and levofloxacin (LVX) 5 µg, (VII) sulfonamides: trimethoprim/sulfamethoxazole (SXT) 25 µg, as categorized by Hassan et al. [17], (VIII) macrolides: erythromycin (E) 15 µg, (IX) lincosamide: clindamycin (CLI) 2 µg, (X) glycopeptides: vancomycin (VAN) 30 µg, (XI) lipopeptide: daptomycin (DAP) 30 µg, (XII) monobactams: aztreonam (AZT) 30 µg, and (XIII) drugs against mycobacteria: rifampin (RIF) 5 µg. The antimicrobial resistance of S. aureus isolates was examined against erythromycin, oxacillin, cefoxitin, clindamycin, daptomycin, vancomycin, and rifampin, while that of P. aeruginosa isolates was tested against ceftazidime, gentamicin, amikacin, aztreonam, cepfime, ciprofloxacin, levofloxacin, and imipenem. The Enterobacteriaceae including E. coli and K. pneumoniae were tested for ampicillin, amoxicillin/clavulanic acid, gentamicin, ciprofloxacin, levofloxacin, ceftriaxone, ertapenem, imipenem, and trimethoprim/sulfamethoxazole, as recommended by the CLSI (2014) [18].

2.5. X-Ray Exposure. All the studied patients were exposed to X-ray by radiography (at kV133 and 5 milliambers) for monitoring the healing of the fracture. The duration of exposure was the same duration of routine X-ray examination (approximately 15–30 ms). The number of X-ray exposure was one, two, and three times. The three exposures were enough to kill all isolated bacteria in the current study. The interval between each exposure was ten days. After each one of exposure, wound swab culture was repeated for screening for bacterial growth. The timing of the wound swab was noted after the second day of X-ray exposure.

3. Results

3.1. Demographical Data. Throughout the study, fifty-one patients suffering from infected open fractures were admitted into the hospital. The age of patients was ranged from 20 to 46 years with mean and standard deviation of 31.7 ± 5.97 years. According to gender, 29 (56.9%) patients were male and 22 (43.1%) patients were female. All studied fractures were located on the lower limb with severity grades of II, IIIA, IIB, and IIIC according to the Gustilo and Anderson classification. All the studied patients were sent for culture, and all of them (51, 100%) showed bacterial growth. The open fractures have become sterile, and no
Pseudomonas aeruginosa 20%
Klebsiella pneumoniae 14%
E. coli 25%
Staphylococcus aureus 41%

Figure 1: Distribution of bacterial isolates from open fractures (n = 51).

Table 2: Antimicrobial susceptibility pattern of S. aureus and P. aeruginosa isolated from infected open fractures.

| Antibiotic               | S. aureus (n = 21) | P. aeruginosa (n = 10) |
|--------------------------|--------------------|------------------------|
| Erythromycin             | 19 (90.5%)         | Ceftazidime            |
| Oxacillin                | 21 (100%)          | Gentamicin             |
| Cefoxitin                | 18 (85.7%)         | Amikacin               |
| Clindamycin              | 20 (95.2%)         | Aztreonam              |
| Daptomycin               | 18 (85.7%)         | Cefepime               |
| Vancomycin               | 16 (76.2%)         | Ciprofloxacin          |
| Rifampin                 | 5 (23.8%)          | Levofoxacin            |
| Resistance to 6 antibiotic classes | 16 (76.2%) | Resistance to 4 antibiotic classes | 10 (100%) |
| Multidrug resistance (MDR) | 21 (100%) | Multidrug resistance (MDR) | 10 (100%) |

3.2. Bacterial Isolation. The bacterial isolate distribution is shown in Figure 1. According to these outcomes, S. aureus isolates came in the lead with 21 (41.2%) isolates among all isolated bacteria. On the other side, Gram-negative isolates formed together around 30 (59.8%) isolates and were divided as follows: E. coli with 13 (25.5%) isolates, P. aeruginosa with 10 (19.6%) isolates, and finally, K. pneumoniae with 7 (13.7%) isolates.

3.3. Antimicrobial Susceptibility Patterns. The result illustrated in Table 2 shows a high resistance level of S. aureus and P. aeruginosa isolates to most of the tested antimicrobials. S. aureus isolates showed high resistance patterns to oxacillin (21, 100%), clindamycin (20, 95.2%), erythromycin (19, 90.5%), cefoxitin (18, 85.7%), daptomycin (18, 85.7%), and vancomycin (16, 76.2%). A lower percentage of resistance recorded to rifampin with 5 (23.8%). Methicillin-resistant S. aureus (MRSA) isolated with isolation rate of 85.7% (18 isolates) depended on the resistance pattern of cefoxitin and oxacillin. All isolated S. aureus (21, 100%) showed a multidrug resistance (MDR) pattern. Most of MDR S. aureus isolates (16, 76.2%) showed resistance to six antibiotic classes.

The results of antimicrobial susceptibility test for P. aeruginosa isolates revealed resistance for most tested antibiotics including ceftazidime (9, 90%), cefepime (9, 90%), gentamicin (8, 80%), amikacin (8, 80%), aztreonam (7, 70%), and imipenem (7, 70%). Low level of resistance recorded against ciprofloxacin (3, 30%) and levofloxacin (3, 30%). All P. aeruginosa (10, 100%) isolates showed MDR pattern. Seven (70%) of P. aeruginosa isolates showed resistance to four antibiotic classes, as shown in Table 2.

In the study, the outcomes of susceptibility pattern for both E. coli and K. pneumoniae isolates showed resistance for all studied antibiotics except ciprofloxacin and levofloxacin which have low percentage of resistance (3 (23.1%) for E. coli and 2 (28.6%) for K. pneumoniae), as shown in Table 3. All isolated E. coli and K. pneumoniae showed resistance to ampicillin and gentamicin (13 (100%) and 7 (100%), respectively). High resistance patterns among E. coli isolates were reported against amoxicillin/clavulanic acid (11, 84.6%), ceftriaxone (11, 84.6%), ertapenem (10, 76.9%), trimethoprim/sulfamethoxazole (10, 76.9%), and imipenem (9, 69.3%). The resistance pattern of K. pneumoniae showed high resistance to amoxicillin/clavulanic acid, ceftriaxone, and trimethoprim/sulfamethoxazole with 85.7% (6 isolates) and to ertapenem and imipenem with 71.4% (5 isolates). All isolated E. coli (13, 100%) and K. pneumoniae (7, 100%) isolates showed resistance to amoxicillin/clavulanic acid (13 (100%)) and ertapenem (13, 100%).

Table 3: Antimicrobial susceptibility pattern of E. coli and K. pneumoniae isolated from infected open fractures.

| Antibiotic                        | E. coli (n = 13) | K. pneumoniae (n = 7) |
|-----------------------------------|-----------------|----------------------|
| Ampicillin                        | 13 (100%)       | 7 (100%)             |
| Amoxicillin/clavulanic acid       | 11 (84.6%)      | 6 (85.7%)            |
| Gentamicin                        | 13 (100%)       | 7 (100%)             |
| Ciprofloxacin                     | 3 (23.1%)       | 2 (28.6%)            |
| Levofloxacin                      | 3 (23.1%)       | 2 (28.6%)            |
| Ceftriaxone                       | 11 (84.6%)      | 6 (85.7%)            |
| Ertapenem                         | 10 (76.9%)      | 5 (71.4%)            |
| Imipenem                          | 9 (69.3%)       | 5 (71.4%)            |
| Trimethoprim/sulfamethoxazole     | 10 (76.9%)      | 6 (85.7%)            |
| Multidrug resistance (MDR)        | 13 (100%)       | 7 (100%)             |
| Resistance to 5 antibiotic classes| 10 (76.9%)      | 5 (71.4%)            |
3.4. Bacterial Isolates and Grades of Open Fractures. The relationship between bacterial isolates from preliminary culture and grades of open fractures was studied statistically using Fisher’s exact test, as reported in Table 4. The results showed a statistically significant difference in the type of bacterial isolates among grades of open wound fractures (p < 0.001). Most of S. aureus isolates were isolated from grade II open fractures with isolation rate reached 95.2% (20 isolates). On the other side, Gram-negative bacteria came in the foreground among grade III classes of open fractures. The isolation rate of P. aeruginosa was high among grade IIIA with 6 (60%) and that of K. pneumoniae was higher in grade IIIB with 5 (71.4%). However, grade IIC open wound fractures showed that the higher isolation of bacteria was recorded for E. coli isolates with incidence rate of 69.2% (9 isolates).

3.5. Number of X-Ray Exposure. An additional correlation was done between the X-ray exposure number and the type of causative bacteria to find the best X-ray exposure number for each one. The result of this comparison showed a statistically high significant relationship for all types of causative bacteria with a p value <0.001. The outcomes showed that the perfect time of killing S. aureus was one X-ray exposure with incidence rate of 85.7% (18 isolates), whereas P. aeruginosa and K. pneumoniae were dead after the second exposure of X-ray with incidence rate of 80% (8 isolates) and 100% (7 isolates), respectively. On the other side, the optimum times of X-ray exposure to E. coli were between two and three times of exposure with incidence rates of 46.1% (6 isolates) and 53.9% (7 isolates), respectively, as shown in Table 5.

3.6. Number of X-Ray Exposure and Grades of Open Fractures. In the current study, the severity of the studied fractures was arranged between grades II and III according to the Gustilo and Anderson classification. Grade II was reported in 21 (41.2%) fractures and grade III was reported in 30 (58.8%) fractures distributed on IIIA, IIIB, and IIC with 10 (19.6%) for each one, as shown in Table 6.

Fisher’s exact test was utilized to measure the relationship between the number of X-ray exposure and severity (grades) of open fractures against each type of bacteria. The results showed a statistically high significant relationship with a p value <0.001. Infected open fractures with grade II showed sterile culture media after the first time of X-ray exposure, and these wounds were infected with S. aureus or P. aeruginosa with 18 (90%) and 1 (100%), respectively. As for infected open fractures with the grade IIIC, these wounds showed sterile culture media after the third time of X-ray exposure, and the causative agent of wound infection was E. coli or P. aeruginosa with incidence rates of 77.8% (7 isolates) and 100% (1 isolate), respectively. On the other side, all E. coli, P. aeruginosa, and K. pneumoniae isolates that caused open fracture infections with grades IIIA and IIIB showed sterile culture media after the second time of X-ray exposure with an incidence rate of 100% for each type of bacteria, as mentioned in Table 6.

From the above, we concluded that Gram-positive S. aureus was killed after the first time of X-ray exposure, while Gram-negative bacterial isolates (P. aeruginosa and K. pneumoniae) were dead after the second time of X-ray exposure, and most E. coli isolates were killed after the third time of X-ray exposure.

4. Discussion

For several years, prevention and controlling infection for open fractures stays the crucial goal in the treatment of these wounds, so different methods were applied to achieve this goal [19, 20]. All these methods are depending on utilizing different types of antibiotic regimes [21, 22]. These antibiotic patterns benefit in several cases, but when the causative bacterial agents were multidrug resistance as shown in this study, these methods become ineffective in the treatment of

---

**Table 4**: Grades of open fractures according to the Gustilo and Anderson classification among four types of bacteria isolated by the study.

| Type of bacteria            | Grade of open fractures | Total n (%) |
|-----------------------------|-------------------------|-------------|
|                             | II n (%) | IIA n (%) | IIIB n (%) | IIC n (%) |
| **Staphylococcus aureus**   | 20 (95.2%) | 1 (4.8%) | 2 (20%) | 1 (10%) | 21 (100%) |
| **Pseudomonas aeruginosa**  | 1 (10%) | 6 (60%) | 2 (20%) | 9 (69.2%) | 13 (100%) |
| **Escherichia coli**        | — | 1 (7.7%) | 3 (23.1%) | — | 4 (100%) |
| **Klebsiella pneumoniae**   | — | 2 (28.6%) | 5 (71.4%) | — | 7 (100%) |

*p value = <0.001 (very high significant). *p value = Fisher’s exact test with confidence interval of 99%.

**Table 5**: Times of X-ray exposure required to kill four types of isolated bacteria by the study.

| Type of bacteria            | Times of X-ray exposure | Total n (%) |
|-----------------------------|-------------------------|-------------|
|                             | One n (%) | Two n (%) | Three n (%) |
| **Staphylococcus aureus**   | 18 (85.7%) | 2 (9.5%) | 1 (4.8%) | 21 (100%) |
| **Pseudomonas aeruginosa**  | 1 (10%) | 8 (80%) | 1 (10%) | 10 (100%) |
| **Escherichia coli**        | — | 6 (46.1%) | 7 (53.9%) | 13 (100%) |
| **Klebsiella pneumoniae**   | — | 7 (100%) | — | 7 (100%) |

*p value = <0.001 (very high significant). *p value = Fisher’s exact test with confidence interval of 99%. 

showed MDR pattern. 10 (76.9%) of E. coli and 5 (71.4%) of K. pneumoniae isolates showed resistance to 5 antibiotic classes.
open fracture infections. This problem prompted us to research other ways of treating infected open fractures.

The current study was carried out on open fractures at the lower limb because the development of infectious complexity has a greater danger at the lower limb fractures as reported by prior studies [23, 24]. Additionally, the study was conducted on open fractures arranged in severity between grades II and IIIA, B, and C, because these grades are most susceptible to develop the infection [25, 26].

The reported findings of this study demonstrated that the most common bacteria isolated from studied open fractures were *S. aureus* and Gram-negative bacteria include *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. These results were identical to those recorded by Bratzler et al. in 2013 [27]. *S. aureus* recorded the highest isolation rate close to that of other studies with 48.4% and 36% of *S. aureus* isolates [28, 29]. Among isolated *S. aureus* of open fractures, the higher portion was recorded for MRSA isolates, and the high percentage of MRSA was also reported by Latha and Jain et al. with isolation rate of 57.3% and 63.29%, respectively [28, 30]. Gram-negative bacteria isolated from open fractures with isolation rate higher than the result of another study that recorded 33.34% as an isolation rate [31]. *E. coli* isolates recorded the highest isolation rate among Gram-negative bacteria, while another study showed a lower isolation rate of 13% [29]. In our study, the isolation rate of *P. aeruginosa* was close to that recorded by another study 26.3% [28]. *K. pneumoniae* isolation rate was slightly higher than the result of another study (9%) [29].

The results of antimicrobial susceptibility test showed MDR for all isolated bacterial species, as mentioned in Tables 2 and 3. In the study, the most isolated bacteria from open fractures showed resistance toward at least four classes of antibiotics, as demonstrated by Hassan et al. [17]. Most of *S. aureus* were resistant to six antibiotic classes, and this resistance pattern of *S. aureus* also reported by another study with 30.1% [32].

The results of open fractures with grade II showed infection by *S. aureus* with highest isolation rate, as was reported previously by another study [4], while open fractures with grade III showed infection with Gram-negative bacteria including *E. coli*, *P. aeruginosa*, and *K. pneumoniae* as reported by the previous study [32].

In the present study, we showed all studied infected wound fractures affected by X-ray, and this was confirmed by bacterial culture after each time of exposure. This effect of X-ray returns to the X-ray interaction with matter to produce unstable ions and free electrons. Furthermore, these free electrons may react with other atoms, which could break the DNA molecules and cause mutations. On the other side, X-ray irradiation of biological material forms reactive hydroxyl radicals, and this leads to DNA damage and other cellular macromolecules and causes cell death [33].

X-ray exposure appeared effective on *S. aureus* from the first time of exposure, while Gram-negative bacteria (*P. aeruginosa* and *K. pneumoniae*) showed effectiveness after the second time of X-ray exposure. This may return to the differences in the cell wall structure of Gram-positive and Gram-negative bacteria. The cell wall of Gram-positive bacteria has a larger amount of proteins (peptidoglycan) than that of Gram-negative bacteria [34], and X-ray has more effect on different prokaryotes by oxidative protein damage (protein carbonylation), and this leads to inactivation in the specific enzymes required in DNA repair and replication [35, 36]. In addition, X-ray radiation contains photoelectrons and Auger electrons, which damage the double-stranded DNA [37].

| Type of bacteria          | Times of X-ray exposure |  |   |   |   |
|---------------------------|-------------------------|---|---|---|---|
|                           | One n (%)               | Two n (%)  | Three n (%) | Total n (%) |
|                           |                         |            |             |             |
| *Staphylococcus aureus*   | Grade IIA               | —           | —           | 1 (100%)    |
|                           | Grade IIB               | —           | —           | 2 (100%)    |
|                           | Grade III               | —           | —           | 1 (100%)    |
|                           | Total                   | —           | —           | 21 (100%)   |
| *Pseudomonas aeruginosa*  | Grade IIA               | —           | 6 (100%)   | 10 (100%)   |
|                           | Grade IIB               | —           | 2 (100%)   | 10 (100%)   |
|                           | Grade III               | —           | —           | 1 (100%)    |
|                           | Total                   | —           | —           | 10 (100%)   |
| *Escherichia coli*        | Grade IIA               | —           | 1 (100%)   | 7 (77.8%)   |
|                           | Grade IIB               | —           | 3 (100%)   | 9 (69.2%)   |
|                           | Total                   | —           | 2 (22.2%)  | 13 (100%)   |
| *Klebsiella pneumoniae*   | Grade IIA               | —           | 2 (100%)   | 2 (28.6%)   |
|                           | Grade IIB               | —           | 5 (100%)   | 5 (71.4%)   |
|                           | Total                   | —           | 7 (100%)   | 7 (100%)    |

* p value of total = <0.05 (highly significant). *p value = Fisher’s exact test with confidence interval of 99%.

---

Table 6: Times of X-ray exposure and grades of open fractures according to the type of isolated bacteria in the study.
The controversial results of \textit{E. coli} isolates showed that half of \textit{E. coli} isolates were killed after the second exposure of X-ray, and the other half were killed after the third exposure; this may return to the ability of some \textit{E. coli} isolates to develop ionizing radiation resistance by the ability to tolerate DNA damage \cite{38}.

### 5. Conclusion

In this study, we concluded that X-ray exposure shows high effectiveness in treating infected open fractures. Gram-positive \textit{S. aureus} was the most causative agent for grade II fractures, and they were killed after the first time of X-ray exposure, while Gram-negative bacteria were the most causative agent for grade III fractures. In grade IIA and IIB fractures, the causative agents were killed after two times of X-ray exposure. In grade IIC fractures, the bacterial agents were killed after three times of X-ray exposure.

### Data Availability

The data used to support the findings of this study are included within the article.

### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

### References

[1] W. W. Cross III and M. F. Swiontkowski, “Treatment principles in the management of open fractures,” \textit{Indian Journal of Orthopaedics}, vol. 42, no. 4, pp. 377–386, 2008.

[2] P. D. Hull, S. C. Johnson, D. J. G. Stephen, H. J. Kreder, and R. J. Jenkinson, “Delayed debridement of severe open fractures is associated with a higher rate of deep infection,” \textit{The Bone & Joint Journal}, vol. 96-B, no. 3, pp. 379–384, 2014.

[3] W. S. Hoff, J. A. Bonadies, R. Cachecho, and W. C. Dorlac, “East practice management guidelines work group: update to practice management guidelines for prophylactic antibiotic use in open fractures,” \textit{The Journal of Trauma: Injury, Infection and Critical Care}, vol. 70, no. 3, pp. 751–754, 2011.

[4] C. G. Zalavras, R. E. Marcus, L. S. Levin, and M. J. Patzakis, “Management of open fractures and subsequent complications,” \textit{The Journal of Bone & Joint Surgery}, vol. 89, no. 4, pp. 884–895, 2007.

[5] S. H. Thomas, A. O. Arthur, Z. Howard, M. L. Shear, J. L. Kadzielski, and M. S. Vrahas, “Helicopter emergency medical services crew administration of antibiotics for open fractures,” \textit{Air Medical Journal}, vol. 32, no. 2, pp. 74–79, 2013.

[6] J. W. Zumsteg, C. S. Molina, D. H. Lee, and N. D. Fappas, “Factors influencing infection rates after open fractures of the radius and/or ulna,” \textit{The Journal of Hand Surgery}, vol. 39, no. 5, pp. 956–961, 2014.

[7] W. D. Lack, M. A. Karunakar, M. R. Angerame et al., “Type III open tibia fractures: immediate antibiotic prophylaxis minimizes infection,” \textit{Journal of Orthopaedic Trauma}, vol. 29, no. 1, pp. 1–6, 2014.

[8] M. de Castro Fernandes, L. R. Peres, A. C. de Queiroz Neto, J. Q. Lima Neto, F. M. Turibio, and M. H. Matsumoto, “Open fractures and the incidence of infection in the surgical debridement 6 hours after trauma,” \textit{Acta Ortopédica Brasileira}, vol. 23, no. 1, pp. 38–42, 2015.

[9] C. L. O’Brien, M. Menon, and N. M. Jomha, “Controversies in the management of open fractures,” \textit{The Open Orthopaedics Journal}, vol. 8, no. 1, pp. 178–184, 2014.

[10] C. C. Savelli, R. W. Belknap, S. J. Morgan, and C. S. Price, “The role of prophylactic antibiotics in open fractures in an era of community-acquired meticillin-resistant \textit{Staphylococcus aureus},” \textit{Orthopedics}, vol. 34, no. 8, pp. 611–616, 2011.

[11] M. J. Patzakis and J. Wilkins, “Factors influencing infection rate in open fracture wounds,” \textit{Clinical Orthopaedics and Related Research}, vol. 243, pp. 36–40, 1989.

[12] G. C. Velmahos, K. G. Toutouzas, G. Sarkisyan et al., “Severe trauma is not an excuse for prolonged antibiotic prophylaxis,” \textit{Archives of Surgery}, vol. 137, no. 5, pp. 537–541, 2002.

[13] A. Simon-Deckers, E. Brun, B. Gouget, M. Carrière, and C. Sicard-Roselli, “Impact of gold nanoparticles combined to X-ray irradiation on bacteria,” \textit{Gold Bulletin}, vol. 41, no. 2, pp. 187–194, 2008.

[14] R. S. Norman, J. W. Stone, A. Gole, C. J. Murphy, and T. L. Sabo-Attwood, “Targeted photothermal lysis of the pathogenic bacteria, \textit{Pseudomonas aeruginosa}, with gold nanorods,” \textit{Nano Letters}, vol. 8, no. 1, pp. 302–306, 2008.

[15] Y. Luo, M. Hossain, C. Wang et al., “Targeted nanoparticles for enhanced X-ray radiation killing of multidrug-resistant bacteria,” \textit{Nanoscale}, vol. 5, no. 2, pp. 687–694, 2013.

[16] J. F. McFadden, \textit{Biochemical Tests for Identification of Medical Bacteria}, Lippincott Williams and Wilkins, vol. 2, pp. 15–38, Philadelphia, PA, USA, 3rd edition, 2000.

[17] M. A. Hassan, T. M. Tamer, A. A. Rageh, A. M. Abou-Zeid, E. H. F. Abd El-Zaher, and E.-R. Kenawy, “Insight into multidrug-resistant microorganisms from microbial infected diabetic foot ulcers,” \textit{Diabetes & Metabolic Syndrome: Clinical Research & Reviews}, vol. 13, no. 2, pp. 1261–1270, 2019.

[18] Clinical and Laboratory Standards Institute (CLSI), \textit{Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fourth Informational Supplement: CLSI Document M100-S24}, Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2014.

[19] W.-J. Metsemakers, J. Onsea, E. Neutjens et al., “Prevention of fracture-related infection: a multidisciplinary care package,” \textit{International Orthopaedics}, vol. 41, no. 12, pp. 2457–2469, 2017.

[20] WHO, \textit{Global Guidelines for the Prevention of Surgical Site Infection}, World Health Organization, Geneva, Switzerland, 2016.

[21] K. Samai and A. Vilella, “Update in therapeutics,” \textit{Journal of Trauma Nursing}, vol. 25, no. 2, pp. 83–86, 2018.

[22] H. R. Shorin, M. G. Daghi, M. Mirkazemi et al., “Antibiotic prophylaxis in bacterial infection of type IIIA open fracture of tibial shaft with or without fibula fracture,” \textit{Razavi International Journal of Medicine}, vol. 4, no. 2, Article ID e57811, 2016.

[23] D. Weber, S. K. Dulai, J. Bergman, R. Buckley, and L. A. Beaupre, “Time to initial operative treatment following open fracture does not impact development of deep infection: a prospective cohort study of 736 subjects,” \textit{Clinical Orthopaedics and Related Research}, vol. 28, no. 11, pp. 613–619, 2014.

[24] F. Moola, A. Carli, G. Berry, R. Reindl, D. Jacks, and E. Harvey, “Attempting primary closure for all open fractures: the effectiveness of an institutional protocol,” \textit{Canadian Journal of Surgery}, vol. 57, no. 3, pp. E82–E88, 2014.

[25] C. G. Zalavras, R. E. Marcus, L. S. Levin, and M. J. Patzakis, “Management of open fractures and subsequent complications,” \textit{Instructional Course Lectures}, vol. 57, pp. 51–63, 2008.
A. N. Pollak, A. L. Jones, R. C. Castillo, M. J. Bosse, and E. J. MacKenzie, “The relationship between time to surgical débridement and incidence of infection after open high-energy lower extremity trauma,” *The Journal of Bone and Joint Surgery-American Volume*, vol. 92, no. 1, pp. 7–15, 2010.

D. W. Bratzler, E. P. Dellinger, K. M. Olsen et al., “Clinical practice guidelines for antimicrobial prophylaxis in surgery,” *American Journal of Health-System Pharmacy*, vol. 70, no. 3, pp. 195–283, 2013.

T. Latha, B. Anil, H. Manjunatha et al., “MRSA: the leading pathogen of orthopedic infection in a tertiary care hospital, south India,” *African Health Sciences*, vol. 19, no. 1, pp. 1393–1401, 2019.

M. Sisay, T. Worku, and D. Edessa, “Microbial epidemiology and antimicrobial resistance patterns of wound infection in Ethiopia: a meta-analysis of laboratory based cross-sectional studies,” *BMC Pharmacology and Toxicology*, vol. 20, no. 35, 2019.

D. Agarwal, R. Maheshwari, A. Agrawal, V. Chauhan, and A. Juyal, “To study the pattern of bacterial isolates in open fractures,” *Journal of Orthopedics, Traumatology and Rehabilitation*, vol. 8, no. 1, pp. 1–5, 2015.

G. Godebo, G. Kibru, and H. Tassew, “Multidrug-resistant bacterial isolates in infected wounds at jimma university specialized hospital, Ethiopia,” *Annals of Clinical Microbiology and Antimicrobials*, vol. 12, no. 17, 2013.

J. Krishman, B. W. M. Cook, T. J. Schrader, and S. Theriault, “Evaluation of the effects of radiation from an X-ray baggage inspection system on microbial agents,” *Applied Biosafety*, vol. 15, no. 1, pp. 9–14, 2010.

H. Carsenti-Etesse, F. Doyon, N. Desplaces et al., “Epidemiology of bacterial infection during management of open leg fractures,” *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 18, no. 5, pp. 315–323, 1999.

T. J. Silhavy, D. Kahne, and S. Walker, “The bacterial cell envelope,” *Cold Spring Harbor Perspectives in Biology*, vol. 2, no. 5, Article ID a000414, 2010.

M. J. Daly, “Death by protein damage in irradiated cells,” *DNA Repair*, vol. 11, no. 1, pp. 12–21, 2012.

A. Krisko and M. Radman, “Protein damage and death by radiation in *Escherichia coli* and *Deinococcus radiodurans*,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 32, pp. 14373–14377, 2010.

S. K. Sahu, Z. P. Kortylewicz, J. Baranowska-Kortylewicz, R. A. Taube, S. J. Adelstein, and A. I. Kassis, “Strand breaks after the decay of iodine-125 in proximity to plasmid pBR322 DNA,” *Radiation Research*, vol. 147, no. 4, pp. 401–408, 1997.

D. R. Harris, S. V. Pollock, E. A. Wood et al., “Directed evolution of ionizing radiation resistance in *Escherichia coli*,” *Journal of Bacteriology*, vol. 191, no. 16, pp. 5240–5252, 2009.