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RESEARCH ARTICLE

The Prevalence of the Gram Positive and Gram Negative Bacteria in Open Fractures and their Resistance Profiles to Antimicrobial Agents

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Abstract

Objectives: We aimed to evaluate the bacteriology of open fractures and to determine the susceptibility patterns of the identified bacterial species to some antimicrobial agents. Methods: 150 superficial swabs were collected from open fracture wounds of patients visited private fractures clinics in Al- Diwaniya city/ Iraq in a period from January to December 2016. Bacterial species recovered from the collected samples were identified by cultural, morphological and biochemical characteristics, then confirmed using Vitek2 compact system. Susceptibility to antimicrobial agents was determined using disc diffusion method. Results: A total of 119 bacterial isolates were obtained from culture positive fracture wounds. The most frequent identified bacterial species was *Staphylococcus aureus* (23.52%) followed by *Acinetobacter spp* (19.32%) ,then *E.coli* (14.28%), *Pseudomonas spp* (11.76%), *Enterobacter spp* (9.24%), Klebsiellaspp (6.72%), Coagulase Negative Staphylococci (5.04%), Citrobacterspp (4.20%), Proteus spp (2.52%), *Diphtheroids* (0.84),*Streptococcus pyogenes* (0.84%), *Viridans streptococci*(0.84%), *Micrococcus* spp (0.84%).Most of isolates (64.70%) were obtained from upper extremities compared with the lower *extremities* (35.29%). The average resistance rate of *Staphylococcus aureus* in the current study was (38.31%), the Coagulase Negative Staphylococci(26.7%), *Diphtheroids* (70%), *Streptococcus pyogenes* (40%), *Viridans streptococci*(20%), and *Micrococcus spp*(10%)., *Acinetobacter spp* (58.36%), *E.coli* (43.58%), *Pseudomonas spp*(62.19%), *Enterobacter spp* (48.21%), *Klebsiella spp* (40%), *Citrobacter spp* (32.5%), *Proteus spp* (35%). Inhibitory effects of citric acid and vinegar on all isolated bacterial species growth were recorded. Conclusion: *S. aureus* and Acinetobacter spp were the predominant bacterial species isolated from open fractures patients with extensive antibiotic resistance patterns for most of isolated bacterial species underscoring the need to monitor the antibacterial resistance pattern routinely in order to select the right prophylaxis and treatment to open fracture wounds infections. The use of citric acid and vinegar is recommended to effectively eliminate multiple antibiotic resistant bacteria in open fractures.

Keywords: *Open fractures, Gram positive bacteria, Gram negative bacteria, Antimicrobial agents.*

Introduction

Open (compound) fractures are defined as fractures that contact with the external environment via the wound, commonly arise from high-energy injury. The major reasons of open fractures involve road accidents, assault, down from a high, shotgun, injury of machine. These fractures are still common, participating to about 4% of all fractures [1]. The main complication of open fracture is infection at the place of injured wounds, which may happen as many as 15% of total wounds. Connect of the fractured bone fractions to the outside environment, fracture acuity, patient co-morbidities, the existence of damaged soft tissue, and the lateness in therapy, collaborate to risk of infection [2]. In severe cases, chronic osteomyelitis, lack of function, or eventually loss of limb may result from deep fracture-site infections [3]. Nearby 70% of

contamination in open fractures wounds happens at the period of trauma from both skin and external environment. In some conditions, the bacterium does not exist at the period of injury, and the wound becomes subsequently infected. The relation between bacterial groups in soft tissue and bone lesions vary widely over time[4]. Depending on the kinds of microbe causing infection compared with those shown on wound culture firstly, open fracture wounds infections classified as nosocomial infections[5].

The plurality of open wounds are caused by polymicrobial aerobic-anaerobic micro flora; and are more virulent which makes healing is delayed, therefore, different antibiotics are considerably and inappropriately prescribed for wound therapy, which frequently lead to the selective pressure of antibiotic-resistant strains, and subsequently increasing of antibiotic resistant strains emergence among wound infections, especially in the hospital environment[6].Wound recovery requires a perfect hygienic environment, one of the significant actions to maintain the process of recovery ongoing to sanitize injured tissue from each microbial contamination[7].

The most favorable topical medication is an equilibrium between microbicidal efficiency and to tolerability. In general, extremely reactive antiseptics are evaluated as too toxic, and some of them intervene with natural healing process. Moreover, frequent and extravagant treatment of wounds with commonly used antiseptic agents, unless short-time purpose, to strike the causal pathogens and control the infection, may lead to unfavorable consequences or support a microenvironment comparable to those establish in chronic wounds[8].The current study is the first study intended to investigate the profile of bacteria exist in open fractures wounds in Al- Diwaniya governorate, their susceptibility to antibiotics and susceptibility to citric acid and vinegar.

Materials and Methods

Specimen's Collection

A total of 150 superficial swabs were collected from patients who clinically diagnosed to have open fractures in private fractures clinics in Al- Diwaniya city/ Iraq in a period from January to December 2016. Age, sex, and hospitalization were recorded for each patient. The swabs were aseptically collected by standard methods as described in [9].

Isolation and Identification

Each swab was streaked on macconkey agar, nutrient agar, and blood agar then incubated at 37°C for 24 hours aerobically and an aerobically for primary isolation. Bacterial isolates were identified using cultural, morphological and biochemical characteristics[9, 10], then confirmed using Vitek2 compact system.

Antibiotic Susceptibility Test

Susceptibility of bacterial isolates to antibiotics were determined by Bauer Kirby's disc diffusion method as recommended by the Clinical and Laboratory Standards Institute[11]. Mueller-Hinton plates were seeded with a 0.5 McFarland standard bacterial suspension, and antibiotic disks were placed, then plates were incubated at 37°C for 24 hours. Diameters of inhibition zone were measured and compared with the guidelines supplied by CLSI (2011) [11].

Susceptibility to Citric Acid and Vinegar

Susceptibility of bacterial isolates to citric acid and vinegar was determined by agar well diffusion method. Firstly, stock solutions were prepared (1% W/V i.e., 0.1g citric acid dissolved in sterile distilled water to prepare the final volume of 10ml), and (1% V/V i.e., 0.1ml vinegar dissolved in sterile distilled water to prepare the final volume of 10ml) [12]. 100µl of a 0.5 McFarland standard bacterial suspension was spread thinly on the surface of Muller Hinton agar plates.

Thereafter, 8 mm size wells were made using sterile cork-borer. 100µl of the prepared chemical concentration was poured into a well of inoculated plates. Sterilized distilled water was used as control[13]. After incubation at 37°C for 24 hours, the plates were examined and zone of inhibition were measured and recorded in millimeters[14]then compared to the most effective antibiotic on each bacterial species.

Results and Discussion

Demography

Out of the 150 patients with open fracture wounds, positive cultures have been noted in 96(64%) of males and 35(23.3%) of females, while no bacterial growth have been indicated in 11(7.30%) of males and 8(5.33%) of females as shown in table (1). The results indicated that men (71.30%) are more

affected than women (28.70%), and this is probably due to the involvement of men in some jobs such as machinery processing and construction trades which is in general not acceptable for women in Iraq. This finding is similar to the results of Abraham and Wamisho[15] who found that the male female ratio was 4.8:1**.** In addition, our results revealed that most of open fractures patients were in the productive age groups; distribution of the age groups of patients involved in the current study is shown in Table (2). The highest number of open fracture wounds, 62(41.3%) was recorded in the age group 16-30 years old with positive cultures in 54(36%) and no growth in 8(8.6%). Positive cultures were also yielded with a high number 25(16.6%) from age group31-44 which is the second affected group in the present study 29(19.3%). 24(16%) of patients of this study were ≥15 years old with 21(14%) positive bacterial cultures then 19(12.7%) of the age group 45-59 and 16(10.7%) of patients ≤ 60 with positive culture of 17(11.3%) and 14(9.3%) respectively. The results are similar to the finding of Abraham *et al*[16]that recorded an average age of(31.55%) for patient involved in their study

Table 1: Numbers and percentages of positive and negative cultures of open fracture wounds specimens' basedon patient's gender

Sex	Positive culture		Negative culture		Total	
	Number	%	Number	%	Number	%
Male	96	64	11	7.3	107	71.30
Female	35	23.3	8	5.33	43	28.70
Total	131	87.30	19	12.70	150	100

Table 2: Numbers and percentages of positive and negative cultures of open fracture wounds specimens based on patients age

Age (years)	Positive culture		Negative culture		Total	
	Number	%	Number	%	Number	%
≥ 15	21	14	3	2	24	16
16-30	54	36	8	8.6	62	41.3
31-44	25	16.6	4	2.6	29	19.3
45-59	17	11.3	2	1.3	19	12.7
≤ 60	14	9.3	2	1.3	16	10.7
Total	131	87.3	19	12.7	150	100

Etiologic Agents

Table (3) shows the bacterial species identified from the upper and lower extremities of open fractures. Most of isolates 77 (64.70%) were obtained from upper extremities compared with the lower extremities 42(35.29%).A total of 119 bacterial isolates were gotten from culture positive fracture wounds. The predominant species was Staphylococcus aureus (23.52%).This is similar to previous reports in multiple places in Ethiopia [15,17-19].*S. aureus* is normally found in hospitals environment increasing the risk of fracture wounds cross infections in patients admitted to these places, and it is a normal flora species of the skin of healthy people that can disseminate easily to soft tissues when the skin gets break[20, 21].*Acinetobacter spp* was the second frequent identified bacterial species in this study with a percentage of (19.32%). The results are similar to investigation of infections of war associated fractures in USA[22] as well as in Ethiopia[15]. The other identified bacterial species in the present study were :*E.coli* (14.28%), *Pseudomonas spp* (11.76%), *Enterobacter spp*(9.24%), *Klebsiella spp* (6.72%), Coagulase Negative Staphylococci (5.04%), *Citrobacter spp* (4.20%), *Proteus spp* (2.52%), *Diphtheroids* (0.84) ,*Streptococcus pyogenes* (0.84%), *Viridans streptococci*(0.84%), *Micrococcus spp* (0.84%).

Table 3:Bacterial species isolated from the upper and lower extremities of open fractures

Type of bacteria	upper extremities		Lower extremities		Total	
	No.of isolates	%	No.of isolates	%	No.of isolates	%
Staphylococcus aureus	17	14.28	11	9.24	28	23.52
Acinetobacter spp	15	12.60	8	6.72	23	19.32
E.coli	11	9.24	6	5.04	17	14.28
Pseudomonas spp	9	7.56	5	4.20	14	11.76
Enterobacter spp	7	5.88	4	3.36	11	9.24
Coagulase	6	5.04	-	-	6	5.04

Negative Staphylococci						
Klebsiellaspp	5	4.20	3	2.52	8	6.72
Citrobacterspp	3	2.52	2	1.68	5	4.20
Proteus spp	2	1.68	1	0.84	3	2.52
Diphtheroids	1	0.84	-	-	1	0.84
Streptococcus pyogenes	-	-	1	0.84	1	0.84
Viridans streptococci	-	-	1	0.84	1	0.84
Micrococcus spp	1	0.84	-	-	1	0.84
Total	77	64.70	42	35.29	119	100

Antibiotic Resistance Pattern

The antibiotics resistant profiles of the identified gram positive species tested for 10 types of commonly used antimicrobial agents showed that (92.8 %) of *S. aureus* isolates were resistant to ampicillin, (58.2%)to amoxicillin, (46.5%)to ciprofloxacin and tetracycline, (28.5%) to each of amoxicillin clavulanic acid, chloramphenicol, gentamycin, and norfloxacin, (14.3%) to ceftriaxone, and(10.8%) to trimethoprim-sulphamethoxazol. The average resistance rate of *S. aureus* was (38.31%) to most of antibiotics tested.

The results are lower than results of studies that have been performed in Ethiopia [19, 23, 24] where an average of resistant of (52%) up to (75%) was recorded.The Coagulase Negative Staphylococci isolates showed (50%) resistance to ampicillin and amoxicillin, (33.4%) to gentamycin, tetracycline, and ciprofloxacin, (16.7%) to amoxicillin clavulanic acid, chloramphenicol, ceftriaxone, andnorfloxacin; no resistance to all Coagulase Negative Staphylococci isolates was observed to trimethoprim-sulphamethoxazol.

Diphtheroids isolates revealed (100%) resistance to amoxicillin clavulanic acid, gentamycin,amoxicillin, tetracycline,trimethoprim-sulphamethoxazol, ceftriaxone, andnorfloxacin, while no resistanceto ampicillin, chloramphenicol, and ciprofloxacin. *Streptococcus pyogenes* isolates were (100%) resistant to ampicillin, trimethoprim-sulphamethoxazol, ceftriaxone, and norfloxacin, while they were (100%) sensitive to amoxicillin clavulanic acid, chloramphenicol, gentamycin, amoxicillin, and tetracycline. *Viridans streptococci* isolates were (100%) resistant to ampicillin and norfloxacin, and (100%) sensitive to the other types of antibiotics. In addition, *Micrococcus spp* isolate was only resistant (100%) to ampicillin and sensitive to the other antimicrobial agent (Figure 1). The average resistance rate of the Coagulase Negative Staphylococci in the current study was (26.7%), *Diphtheroids* (70%), *Streptococcus pyogenes* (40%), *Viridans streptococci*(20%), and *Micrococcus spp* (10%).The antibiotics resistant profile of the identified gram negative bacterial species tested for 10 types of commonly used antimicrobial agents are illustrated in (Figure 2). *Acinetobacter spp* isolates were(87%) resistant to ampicillin and amoxicillin, (78.3%) to amoxicillin clavulanic acid and ceftriaxone, (52.2 %) to chloramphenicol and norfloxacin,(47.9%)to tetracycline, (43.8%)to gentamycin and ciprofloxacin,(13.1%) to trimethoprim-sulphamethoxazol. *E.coli* isolates were (76.5%) resistant to ampicillin, (64.8%)to tetracycline,(58.9%) to amoxicillin,(53%) to amoxicillin clavulanic acid, (47.1%) to chloramphenicol, (41.2%) to gentamycin and trimethoprim-sulphamethoxazol, (29.5%) to ceftriaxone, and (11.8%)to norfloxacin and ciprofloxacin. *Pseudomonas spp* isolates were (92.9%) resistant to amoxicillin, (85.8%) to amoxicillin clavulanic acid, chloramphenicol and trimethoprim-sulphamethoxazol, (78.5%) to tetracycline, (71.5%) to ampicillin and ceftriaxone, (35.8%) to gentamycin, and (14.3%) to norfloxacin.

Moreover, *Klebsiella spp* showed (100%) resistance to ampicillin and amoxicillin, (50%) to tetracycline, (37.5%) to amoxicillin clavulanic acid and ceftriaxone,(25%) to chloramphenicol, gentamycin, and to trimethoprim-sulphamethoxazol.

Citrobacter spp showed (72.5%) resistance to ampicillin and amoxicillin, (50%) to amoxicillin clavulanic acid, (27.5%) to chloramphenicol, gentamycin, (25%) to tetracycline, trimethoprim-sulphamethoxazol, and ceftriaxone. Furthermore, *Enterobacter spp* showed (100%) resistance to ampicillin and amoxicillin, (72.8%) toamoxicillin clavulanic acid, (45.5%) to chloramphenicol,

trimethoprim-sulphamethoxazol, and ceftriaxone,(27.3%)to gentamycin, and (18.2%) to ciprofloxacin. *Proteus spp* showed (100%) resistance to ampicillin, (75%) to tetracycline, (50%) to chloramphenicol, amoxicillin, and trimethoprim-sulphamethoxazol , and (25%) to amoxicillin clavulanic acid with (100%) sensitivity to gentamycin, ceftriaxone, norfloxacin, and ciprofloxacin. The average resistance rate of *Acinetobacter spp* (58.36%), *E.coli* (43.58%), *Pseudomonas spp* (62.19%), *Enterobacter spp*(48.21%), *Klebsiella spp* (40%), *Citrobacter spp* (32.5%), *Proteus spp* (35%) is comparable with a previous study in Ethiopia[20] where the average resistance rate of *Acinetobacter spp* (42.9%), *E.coli*(30.4%), *Pseudomonas spp*(43.5%), *Klebsiella spp* (47.3%), *Proteus spp* (39.9%), and slightly higher for *Citrobacter spp* (54.3%). Most isolated gram positive and negative species in the current study showed high resistance pattern to the majority of the tested antibiotics, and this is probably because these antimicrobial agents are the common used drugs and similar profiles have been illustrated in multiple reports [20, 25, 26]. Some of these antimicrobial agents were utilized as prophylaxis, and excess use of antibiotics can increase developing organisms resistance[27].

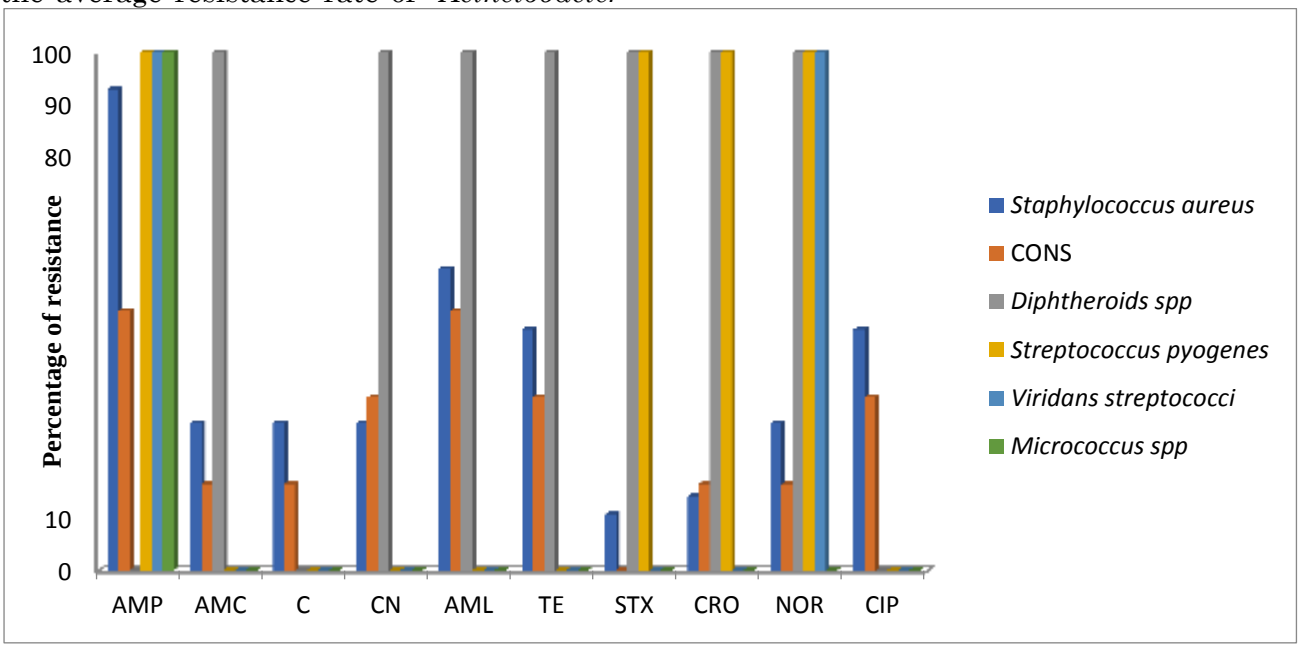

Figure 1:Antibiotic resistance profiles of gram positive bacteria isolated from open fractures

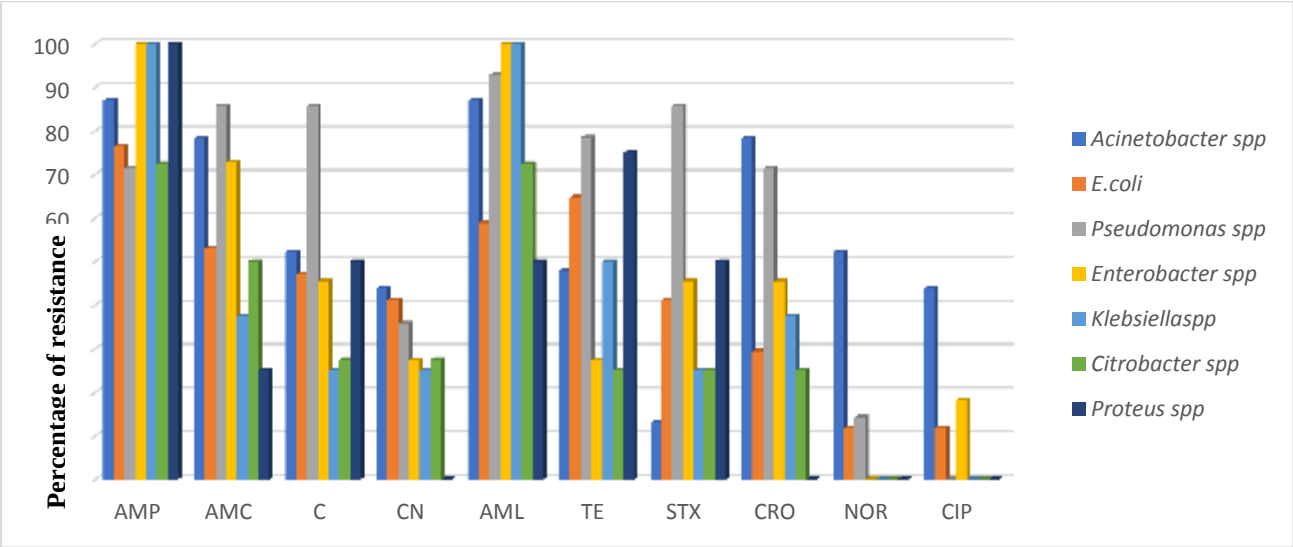

Figure2: Antibiotic resistance profiles of gram negative bacteria isolated from open fractures

CONS: Coagulase Negative Staphylococci, AMP:ampicillin, AMC: amoxicillin clavulanic acid, C: chloramphenicol, CN: gentamycin, AML: amoxicillin, TE: tetracycline, STX: trimethoprim-sulphamethoxazol, CRO: ceftriaxone, NOR: norfloxacin, CIP: ciprofloxacin.

Antimicrobial Effects of Vinegar and Citric Acid on the Growth of the Identified Bacterial Species

High reduction rate on the growth of all isolated bacterial species has been recorded with increase the concentration of citric acid and vinegar with no effects when using 25% dilution (Table 4). Vinegar was more effective (100%) to inhibit the growth of all bacterial species than citric acid, and this is probably due to the antimicrobial activity of the acetic acid of vinegar[28]. Acetic acid is considered as safe under concentrations of 5%, as in vinegar, and did not affect in vivo re epithelialization, but is corrosive in concentrations between 10% and 30% on metal and skin[29]. Antimicrobial activity of acetic acid is PH dependent and referred to direct PH reduction, ionization of the un dissociated acid molecule or alteration of cell membrane permeability [30]. Furthermore, the antiseptic property of citric acid may be referred to reducing the pH that creates an inadequate environment for bacterial growth. It also augments epithelization, which is a main agent in wound healing process [31]. Local antiseptics have usefulness over antibiotics in that their utilization does not promote development of multiple drug resistant strains in hospital environment[8].In addition, the use of organic acids such as citric acid and acetic acid has been recorded as a topical agent for handling bacterial infections of burns, skin and soft tissue[32-34], and the bactericidal and bacteriostatic of citric acid and acetic acid have been illustrated on many types of pathogenic bacteria like Enterobacteriaceae[35], *E. coli*[36], and *Salmonella typhimurium*[37].

Table 4:Antimicrobial effects of vinegar and citric acid on the growth of the identified bacterial species (numbers showed the percentages of susceptibility)

Type of bacteria	Vinegar concentration(V/V) %				Citric acid concentration(W/V) %			
	25	50	75	100	25	50	75	100
Staphylococcus aureus	0	35	47	100	0	45	50	74
Acinetobacter spp	0	21	52	100	0	33	45	71
E.coli	0	32	75	100	0	30	40	65
Pseudomonas spp	0	12	44	100	0	31	50	88
Enterobacter spp	0	35	68	100	0	12	35	71
Coagulase Negative Staphylococci	0	15	42	100	0	13	50	23
Klebsiellaspp	0	8	59	100	0	12	41	59
Citrobacterspp	0	10	50	100	0	15	53	71
Proteus spp	0	13	23	100	0	21	72	82
Diphtheroids	0	100	100	100	0	100	100	100
Streptococcus pyogenes	0	100	100	100	0	100	100	100
Viridans streptococci	0	100	100	100	0	100	100	100
Micrococcus spp	0	100	100	100	0	100	100	100

Conclusion

S. aurous and *Acinetobacter spp* were the major bacterial species isolated from open fractures patients. Extensive antibiotic resistance has been indicated among the gram positive and gram negative isolated bacteria. In addition, citric acid and vinegar may be considered as alternative agents to reduce such infections. The outcome of this report may help to comprehend the bacteriology and epidemiology of open fractures and to reasonably select appropriate antibiotics and prophylactics

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