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Current pathogens infecting open fracture tibia and their antibiotic susceptibility at a tertiary care teaching hospital in South East Asia

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SUMMARY

Background: Tibia fractures represent the most prevalent open long-bone injuries. Indiscriminate, extensive, and unnecessary use of antibiotics has led to the emergence of infections caused by multidrug resistant organisms that increase morbidity and mortality. This study evaluated the spectrum of current organisms infecting the open tibia fractures and their antibiotic susceptibility pattern. This research did not alter the exiting practice of the institute to evaluate the current status.
Methods: This was a cross-sectional study on 628 patients presenting with open fractures of the tibia from July 2018 to July 2020. Sampling for three successive culture (and sensitivity) tests were carried out, 1st on specimens taken in the emergency room (upon patient presentation), 2nd in the emergency theatre after initial debridement, and 3rd in the ward between 12 to 14 days post operatively.
Results: The average age of the patients was 36.2± 15.4 years, with motor vehicle accidents being the predominant aetiology (72.2%). Results of specimen culture demonstrated that debridement could reduce microbial contamination significantly ($P<.05$) from 38.5 % to 26.4%. But from the ward sample, the infection rate was 45.1%, while contamination at entering the ward was only 26.4%. The bacteriological study found predominant multidrug-resistant Gram-negative organisms, namely Pseudomonas spp., *Escherichia coli*, Klebsiella spp., Acinetobacter spp., Enterobacter spp. and Proteus spp. Though Gram-positive *Staphylococcus aureus* was found significantly in the initial culture, they contributed minimally (1.4%) to infect the fracture site.

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Conclusion: The current study found a predominant shift in the trend toward multidrug-resistant Gram-negative organisms in orthopaedic infection, which was accompanied by a worrying pattern of hospital-acquired infection. These results will help to inform future research and policies within our institution.

Introduction

A wound is a breach of soft tissue integrity, even if only the epidermal layer [1]. Breach of soft tissue might lead to exposure of underlying bones or joints to the exterior environment, culminating in an open fracture. The principles of open fracture care are to manage the entire injury while also preventing primary contamination from progressing to a full-fledged infection. [2] The extent of infection rate in open fractures ranges from 0% to 2% for open fractures of Gustilo type I, from 2% to 10% for Gustilo type II, and from 10 % to 50% for fractures of Gustilo type III [3]. In ancient times open fractures had almost deadly consequences requiring urgent amputation. Despite amputation, very few could survive their death without antibiotics, mainly from infection and sepsis [4]. Initial antibiotic therapy is of paramount importance in treating open fractures, and when coupled with early and meticulous debridement, the infection rate can be reduced significantly [5]. Debridement is defined as the removal of necrotic or devitalized tissue from a wound [6].

Effective antimicrobial development over the last century has reduced the incidence of deadly infections, but the development of resistance has obscured its success [7]. The principle of the judicial use of antibiotics and guidelines for controlling infection has been widely published, but guidance is frequently not followed. The World Health Organization has cautioned that antibiotic resistance constitutes a major danger at present and could be a prelude to a post-antibiotic era in which regular illnesses and minor injuries threaten life again [8]. Indiscriminate, extensive, and unnecessary use of antibiotics has led to the development of an increasingly antibiotic-resistant microbial ecosystem and multidrug-resistant (MDR) superinfections worldwide [9].

This infective complication and antibiotic resistance synergistically pose a major threat to the health care system. Updated knowledge about the spectrum of causative organisms, as well as its current resistance pattern, is essential for open fracture management. Tibial shaft fractures account for 2% of all fractures and 44.4% of all open long-bone fractures in adults [10,11]. Due to the specific anatomical features of the tibia (limited soft coverage) more than 15% of its fractures are classified as open and have resulted in being the most infection-prone bone of the body [11]. Considering the open tibia fracture, if we can evaluate the most infection-prone injury as an ideal, it could easily be applied for the less severe one. Hence, the present study has evaluated the current organisms infecting the open tibia fractures and their antibiotic susceptibility pattern. However, this study did not alter the exiting practice of the institute for the evaluation of the current status.

Materials and methods

General information

The present study was held between July 2018 to July 2020 at a tertiary care orthopaedic teaching hospital over patients of both sexes attending the emergency department with open fractures of the tibia. Patients with injury to admission time more than 24 hours, already visible signs of infection, and incomplete/partial antibiotic sensitivity data were excluded. Using a consecutive sampling technique, 685 patients were identified at emergency department, 57 were excluded following exclusion criteria and finally 628 patients were analyzed (Figure 1). In the present study, all first encountered open fractures were considered to be contaminated by pathogens [12]. Wound contamination is the presence of non-proliferating microbes within a wound at a level that does not elicit a host response, while infection occurs when these non-proliferating germs multiply at a pace that elicits a host response [13]. We considered infection clinically by host response with the presence of swelling and increased local temperature, new or increasing pain, pyrexia, purulent discharge, none viable tissue, spreading erythema (cellulitis), abscess, lymphangitis, crepitus, wound dehiscence or delayed healing [2,13].

The Institutional Review Board (IRB) of the National Institute of Traumatology & orthopedic rehabilitation, (NITOR) Dhaka-1207, Bangladesh, approved the research. Informed written consent was received from patients.

Data and specimen collections

After receiving patients' informed written consent for the research participation, data was collected through a standardized data collection form. At the emergency department, demographic variables and mechanism of injury were noted. Because antibiotic prophylaxis is recommended to begin within 3 hours of injury and continue until the first debridement, single doses of prophylactic antibiotics, intravenous penicillin (Flucloxacillin, 500mg) and 3rd generation cephalosporin (Ceftriaxone, 2gm), were given (according to the current practice of the teaching hospital and local antibiotic prophylaxis practices in Indian subcontinent), during the initial resuscitation after ensuring the collection of the first culture sample [14,15]. Patients were sent to the emergency theatre, and debridement was done following the current practice of the teaching institute: using Chlorhexidine, normal saline, hydrogen peroxide, and Povidone-iodine solution. A second post debridement culture sample was obtained, the last saline wash from the wound was delivered and planned for re-debridement within 1–2 days depending on the wound condition. Stable patients were transferred to the post-operative

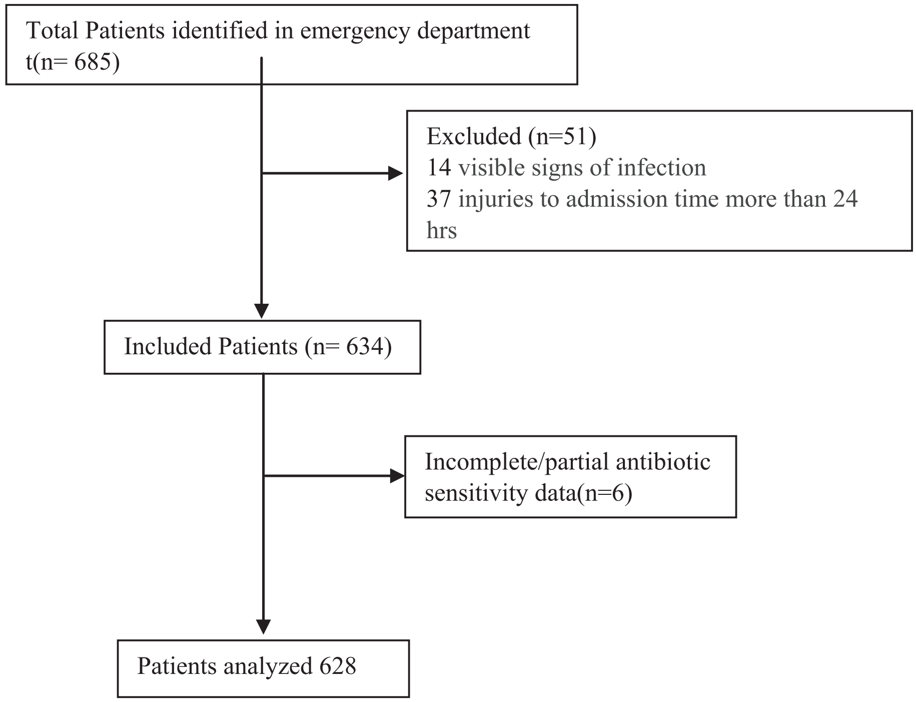

Figure 1. Flow diagram of patients excluded from the study.

ward, followed by to the general ward. Antibiotics were rationalised as per the initial culture and sensitivity results, which were followed further. A third specimen was collected after admission in the ward at 12–14 days and was sent for culture and antibiotic sensitivity. All specimens were collected by single trained data collectors under the supervision of any of the authors available at that time, using a sterile cotton swab in a separate sterile test tube or nutrient broth media.

Identification of bacteria and drug sensitivity test

Specimens were immediately transported to the same microbiology laboratory after collection, and inoculation on Mannitol Salt Agar (MSA), MacConkey Agar (MAC), and Blood Agar (BA) were accomplished within 1 hour. As per previous research, *Staphylococcus aureus* is the most common organism responsible for orthopaedic infections [16]. Mannitol salt agar (MSA) has been used as a selective medium for the isolation of pathogenic staphylococci since 1945; hence, Mannitol Salt Agar (MSA) was also used in addition to MAC and BA [17,18]. Bacterial growth was studied after incubating the culture plates aerobically for 24 hours at 37.0°C. Plates with no growth were kept for additional 24 hours. Colonies were identified by colony morphology, Gram staining and by the conventional biochemical tests such as catalase, coagulase, oxidase, and mannitol fermentation for Gram positive bacteria and urease, indole, citrate, and sugar utilization tests for Gram negative bacteria [19]. The Clinical Laboratory Standards Institute (CLSI) protocol was followed to assess antibiotic susceptibility using the Kirby-Bauer antibiotic testing agar diffusion method. Antibiotic sensitivity was classified as sensitive (S), intermediate (I), or resistant (R) using the standard protocol [19].

Quality assurance

The sterility and function of culture mediums were pretested. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC700603, and *Pseudomonas aeruginosa* ATCC 27853 were used as control bacteria strains in the tests, as per CLSI protocol. A 0.5 McFarland standard barium sulfate ($BaSO_4$) turbidity was used to standardize the density of the inoculum of bacterial suspension [19].

Statistical analysis

The data were tabulated, and quantitative parameters such as the age of patients were summarized in terms of mean with standard deviation and percentage, and paired t-test or χ^2-statistic was used where appropriate. A p-value of <0.05 was considered to be statistically significant, with a 95% confidence interval. Risk factor were analyzed by using multiple logistic regression.

Results

Socio-demographic and injury characteristics

Among 628 studied patients, most (427) were from the 18–40 years age group, in contrast with the least number of patients (46) from the elderly (>60 years) group, and 565 of them were male. Only 291 patients could access emergency care within six hours of injury. According to the Gustilo classification, the Gustilo type III fracture was predominant, 344 (54.8%), followed by type II, 197 (31.4%). About 72.2% of the patients were the victims of RTA, while physical assault, fall from a height, and sports trauma were the subsequent causes (Table I).

Table I
On arrival (at emergency) characteristics of the study subjects (n = 628)

Characteristics	Group	Mean±SD	n
Age (in years)	18 to 40		427 (68.0)
	41 to 60	36.1± 15.4	155 (24.7)
	>60		46 (7.3)
Sex	Male		565 (90.0)
	Female		63 (10.0)
Smoking habit	Yes		161 (25.7)
	No		467 (74.3)
Alcohol consumption	Yes		14 (2.3)
	No		614 (97.7)
Number of co-morbidities	None		264 (42.1)
	1		177 (28.2)
	2		128 (20.3)
	3 or above		59 (9.4)
Mechanism of Injury	RTA		452 (72.2)
	Fall from height		45 (7.2)
	Sports trauma		31 (4.9)
	Physical assault		91 (14.5)
	Others		9 (1.4)
Time elapsed since injury to debridement	Less than 6 hours		246 (39.1)
	6 to 12 hours		248 (39.5)
	More than 12 hours		134 (21.4)
Type of Fracture	Gustilo I		87 (13.9)
	Gustilo II		197 (31.4)
	Gustilo III		344 (54.8)

Values are presented as frequency, mean or percentage.
SD: Standard Deviation.
Percentage in the parenthesis.

Contamination and infection pattern

On arrival, 242 (38.5%) patients had contamination as per the first culture result. Debridement could reduce the contamination significantly ($p < .05$) from 38.5 % to 26.4%. But from the ward sample, the infection rate was 45.1%, while contamination at entering the ward was only 26.4%. (Table II). Patients entering the ward after debridement with no contamination was 73.6% (Table II), but among them, 34.63% became infected (Table III). There was no statistically significant difference ($p > .05$) in the rate of contamination or infection following injury to debridement (admission), whether the debridement was performed before or after six hours, or even after 12 hours (Table IV). In multivariate analysis increasing age, smoking habit, presence of multiple co-morbidities, application of external fixator or wound closure at 1st surgery, failure to cover the wound within five days, higher Gustilo grade and presence of contamination after debridement were significant risk factors for ultimate wound infection. However, injury to debridement (admission) time or alcoholism were not risk factors in multivariate regression analysis (Table V).

Table II
Results of three specimen cultures in terms of organism present/absent among the cases

Organism present or absent	1st culture (on arrival)	2nd culture (at the emergency theater)	3rd culture (from the wards)
Present	242 (38.5 %)	166 (26.4%)	283 (45.1%)
Absent	386 (61.5 %)	462 (73.6%)	191 (30.4%)
Total	628 (100%)	628 (100%)	*474 (75.5%)

The infection rate in the open fracture is 45.1% as per third culture (from the ward).
1. *During the third culture, 154 (24.5%) patients had their wound healed, and the third sample could not be collected.
2. There are significant differences among the three cultures in terms of organisms present or absent among the cases ($p < .05$). (χ2- statistic were employed).
Percentage in the parenthesis.

Bacterial strains identified

The common organisms found in the three cultures were *Staphylococcus aureus,* Pseudomonas spp., *Escherichia coli,* Klebsiella spp., Acinetobacter, Enterobacter spp. and Proteus spp. The number of organisms decreased in the 2nd culture (332 decreased to 232) after debridement but again increased in the 3rd culture (356 in the samples from the ward). Debridement in theatre before ward admission significantly ($p < .05$) reduced the number of organisms, but culture-directed antibiotic therapy after admission in the ward was unable to reduce the organisms significantly ($p > .05$). Furthermore, organisms detected in the third culture were significantly ($p < .05$) different from those found in the culture following debridement. Some of the organisms discovered in post-debridement culture were totally absent in the third, while others were discovered for the first time (Table VI).

Table III
Outcome of contamination to infection/no infection

Outcome	Frequency	Ultimate infection in no contamination and contamination group at ward
No contamination to infection	160 (25.5)	Ultimate infection in no contamination group
No contamination to no infection	302 (48.1)	$= \frac{160}{462} \times 100 = 34.63\%$
		*Total no contamination after debridement =462 (Table II)
Contamination to infection	123 (19.6)	Ultimate infection in contamination group
Contamination to No infection	43 (6.8)	$= \frac{123}{166} \times 100 = 74.09\%$
		*Total Contamination after debridement
		=166 (Table II)
Total	628 (100)	462+166= 628

Percentage in parenthesis.

Antimicrobial susceptibility

Gram-negative organisms were predominant with multidrug resistance. Pseudomonas spp. and Klebsiella spp. were resistant to most of the used antibiotics. The older drug, chloramphenicol demonstrated sensitivity in 55% of *Klebsiella spp*. But *Proteus spp*. was strongly multidrug-resistant (except for imipenem or meropenem). *Enterobacter spp*. has acceptable sensitivity to 4 drugs (imipenem, amikacin, levofloxacin, and chloramphenicol) (Table VII).

Discussion

Nowadays, the discovery of new antibiotics has slowed down [20]. Moreover, the magnitude and extent of traumatic insult are being complicated day by day. Therefore, updated knowledge of current infecting microorganisms with their resistance patterns are essential to increase the expertise of a fracture surgeon. Tibia easily becomes bare following trauma, is very prone to infection, and has become the center of focus for infection study of open fractures [21]. To our knowledge, this is the pioneer paper encompassing the bacterial spectrum of open fractures with their antibiotic susceptibility in the author's country.

The mean age of the study subjects in the present study was 36.2±15.5 years. The young active group being the financial workforce of the society, predominantly suffered open fractures. An epidemiological study on open fracture for 15 years found a similar mean age [22]. Among the study subjects, 345 (89.8%) were male, and 39 (10.2%) were female, which states male predominance. Males are more mobile groups than females for earning the livelihood and prone to injuries because of exposure to risky activities. Various other studies also opined male is more prone to open fracture [22,23]. Regarding the mechanism of injury, road traffic accidents were the predominant mechanism found in 72.9% of cases, and physical assault was the second-highest cause in our series. A recent study on extremity fracture found Road Traffic Accidents (RTA) were responsible in 73.3% of cases [24]. A prospective study from a teaching hospital in Africa and another epidemiological study from the same continent also found road traffic accidents as the most common cause of open fractures followed by interpersonal violence [23,24].

In our series, more than half of the patients (60.9%) failed to reach the hospital for trauma care within 6 hours of injury. A study from São Paulo, Brazil, stated that time elapsed since an injury to admission is very frequently more than 6 hours, resulting of a variety of factors, including the delayed transfer

Table IV
Effect of injury to debridement time on contamination and ultimate infection

Effect on contamination				
Injury to initial debridement	Overall	Contamination	No contamination	*p*-value
Less than 6 hours	246	57	189	.136
More than 6 hours	382	109	273	
Less than 12 hours	494	124	370	.179
More than 12 hours	134	42	92	
Effect on infection				
Injury to initial debridement	Overall	Infection	No infection	*p*-value
Less than 6 hours	189	103	86	.059
More than 6 hours	285	180	105	
Less than 12 hours	379	218	161	.068
More than 12 hours	95	65	30	

p-values obtained using $\chi 2$- statistic.

Table V
Multivariate analysis of the risk factors for infection

Variable	Level	OR	95% CI	*p*-value
Age	1 year increase	1.161	1.013–1.335	0.032
Smoking status	Yes	1.072	1.004–1.147	0.037
Alcoholism	Yes	0.506	0.091–3.034	0.468
Comorbidity	More than one (multiple)	1.663	1.443–1.912	0.001
External Fixation 1st surgery	Yes	1.867	1.017–3.531	0.044
Wound Closed In 1st surgery	Yes	7.851	1.530–52.631	0.014
Time to Irrigation and debridement	Per unit (hour) of delay	0.861	0.560–1.279	0.846
[a]Contamination present after debridement	Yes	3.487	1.295–11.393	0.015
Flap coverage > 5 days	Yes	8.894	2.673–28.674	0.004
Gustilo & Anderson Classification	1 grade increase	1.610	0.997–3.602	0.042

p-values obtained using multiple logistic regression model. Significant at $p<0.05$.
[a] Contamination present after debridement: Positive 2nd Culture.

of patients from primary care or trauma site, management of associated life-threatening injury, and logistical issues, availability emergency operating facilities [25]. As a densely populated country, the transfer of the patient is mostly prolonged in our country. Moreover, a lack of a proper referral system might play a role in the delay. Consequently, only around 40% could have their debridement within 6 hours of injury. However, in our research, injury to debridement time greater than 6 hours, or even greater than 12 hours, was not shown to be a risk factor in either the bivariate or multivariate analyses. A recent systematic review and meta-analysis reported debridement time even up to 24 hours did not affect the infection rate; furthermore, it was found that competent debridement was preferred over rapid and poor debridement [26].

This study found debridement could reduce microbial contamination from 38.5 % to 26.4%. According to EFFORT open reviews, surgical debridement is considered the pivotal and

Table VI
Common Organisms in three Cultures with comparison

	1st culture	2nd culture	*p*-value	2nd culture	3rd culture	*p*-value
	Freq.(%)	Freq.(%)		Freq.(%)	Freq.(%)	
Gram positive						
Staphylococcus aureus	33 (9.9)	10 (4.3%)	[a].013	10 (4.3%)	5 (1.4)	[a].029
Streptococcus spp.	9 (2.7)	8 (3.4)	[a].631	8 (3.4)	8 (2.2)	[a].378
Total Gram positive	42 (12.7)	18 (7.6)	[a].062	18 (7.6)	13 (3.6)	[a].029
Gram negative						
Pseudomonas spp.	65 (19.6)	58 (25.0)	[a].126	58 (25.0)	182 (51.1)	[a].001
Escherichia coli	60 (18.1)	38 (16.4)	[a].603	38 (16.4)	26 (7.3)	[a].005
Klebsiella spp.	56 (16.9)	45 (19.4)	[a].441	45 (19.4)	86 (24.2)	[a].172
Acinetobacter	53 (16.0)	30 (12.9)	[a].317	30 (12.9)	19 (5.3)	[a].001
Enterobacter spp.	28 (8.4)	17 (7.3)	[a].631	17 (7.3)	5 (1.4)	[a].002
Proteus spp.	11 (3.3)	13 (5.6)	[a].183	13 (5.6)	18 (5.1)	[a].791
Citrobacter freundii	9 (2.7)	6 (2.6)	[a].928	6 (2.6)	0 (0%)	-
Serratia spp.	2 (0.6)	1 (0.4)	[a].779	1 (0.4)	0 (0%)	-
Providencia alcalifaciens	4 (1.2)	2 (0.9)	[a].696	2 (0.9)	5 (1.4)	[a].587
Flavobacterium	2 (0.6)	0 (0)	-	0 (0)	0 (0)	-
Plesiomonas spp.	0 (0)	2 (0.9)	-	2 (0.9)	0 (0)	-
Aeromonas	0 (0)	2 (0.9)	-	2 (0.9)	0 (0)	-
Morganella moganii	0 (0)	0 (0)	-	0 (0)	2 (0.6)	-
Total Gram negative	290 (87.34)	214 (92.2)	[a].064	214 (92.2)	343 (96.3)	[a].030
Grand Total	332 (100)	232 (100)	[b].014	232 (100)	356 (100)	[b].368

Though the individual organism types were not different before or after debridement (comparing the1st and 2nd culture, $p > .05$) but debridement could significantly ($p < .05$) reduce the organism load/number of organisms, (comparing the grand total) where culture directed antibiotic therapy after admission in the ward was unable to reduce number significantly ($p > .05$).
Significant ($p < .05$) difference was observed between the total gram-negative and total gram-positive organisms that caused contamination after debridement (2nd culture) and the organism that caused the wound infection (3rd culture). Most of the organisms individually also showed similar significant ($p < .05$) differences. Furthermore, a new organism was identified in 3rd culture, but several that were present in the 2nd culture were absent in the 3rd.
[a] z-test of proportion.
[b] Paired sample t-test was employed to see the difference between organisms.

Table VII
Resistance pattern of common organisms

Organism	Sensitivity	Ampicillin	Amoxacillin	Piperacillin	Cephalaxin	Ceftriaxone	Ceftazidime	Cefepime	Cefixime	Imipenem	Meropenem	Gentamicin	Amikacin	Netilmicin	Doxycyclin	Ciprofloxacin	Levofloxacin	Moxifloxacin	Cotrimoxazole	Chloramphenicol	Azythromycin
Antimicrobial agent's sensitive (S)/Intermediate (I)/Resistance (R) (%)																					
Staphylococcus aureus	S	36.4	36.4	63.6	63.6	48.5	51.5	18.2	15.2	**90.9**	**84.8**	**93.9**	**90.9**	**100**	**84.8**	60.6	63.6	60.6	**90.1**	**90.1**	51.5
	I	00	00	3.0	00	00	12.1	24.2	12.1	00	00	00	00	00	6.1	6.1	9.1	12.1	00	00	00
	R	63.6	63.6	33.3	36.4	51.5	36.4	57.6	72.7	9.1	15.2	6.1	9.1	00	9.1	33.3	27.3	27.3	9.1	9.1	48.5
Escherichia coli	S	6.9	6.9	29.9	18.4	34.5	35.6	41.4	25.3	78.2	**90.8**	74.7	73.6	81.6	54.0	56.3	63.2	51.7	69.0	74.7	18.4
	I	00	00	11.5	00	2.3	3.4	8.0	1.1	11.5	6.9	00	3.4	00	3.4	11.5	2.3	9.2	2.3	00	11.5
	R	93.1	93.1	58.6	81.6	63.2	60.9	50.6	73.6	10.3	2.3	25.3	23.0	18.4	42.5	32.2	34.5	39.1	28.7	25.3	70.1
Pseudomonas spp.	S	1.5	1.5	19.8	10.3	4.2	19.5	31.7	5.3	**58.0**	**50.0**	25.2	45.8	35.9	17.6	38.9	36.3	27.9	20.2	12.6	15.3
	I	00	00	38.2	00	3.1	3.1	0.4	0.8	0.4	7.6	0.4	4.6	11.1	8.4	3.1	5.7	1.9	00	5.7	6.5
	R	98.5	98.5	42.0	89.7	**92.7**	77.5	67.9	**93.9**	41.6	42.4	74.4	49.6	53.1	74.0	58.0	58.0	70.2	**79.8**	**81.7**	**78.2**
Klebsiella spp.	S	00	2.7	15.8	15.8	18.0	24.0	16.4	14.8	**66.1**	**69.9**	39.3	53.6	42.6	39.3	29.0	57.9	34.4	34.4	56.8	8.2
	I	00	00	8.7	00	00	3.8	6.0	00	23.0	10.4	00	2.7	8.2	4.9	23.5	6.6	15.3	0.5	2.2	5.5
	R	100	97.3	**85.4**	**84.2**	**82.0**	72.1	77.6	**85.2**	10.9	19.7	60.7	43.7	49.2	55.7	47.5	35.5	50.3	65.0	41.0	**86.3**
Proteus spp.	S	00	00	50.0	00	00	6.2	6.5	00	**81.3**	**93.8**	18.8	37.5	6.3	6.3	6.3	12.5	6.3	12.5	56.3	12.5
	I	00	00	12.5	00	00	00	00	00	6.3	00	00	6.3	00	6.3	00	6.3	12.5	00	00	00
	R	100	100	37.7	**100**	**100**	**93.8**	**93.5**	**100**	12.5	6.3	**81.3**	56.3	**93.8**	**87.5**	**93.8**	**81.3**	**81.3**	**87.5**	43.8	**87.5**
Acinetobacter	S	00	00	2.9	00	5.9	23.5	17.6	00	20.6	35.3	14.7	29.4	58.8	52.9	26.5	44.1	32.4	38.2	32.4	17.6
	I	00	00	20.6	00	8.8	00	00	00	00	00	00	2.9	8.8	2.9	5.9	11.8	5.9	5.9	2.9	2.9
	R	100	100	76.5	**100**	**85.3**	76.5	**82.4**	**100**	**79.4**	64.7	**85.3**	67.6	32.4	44.1	67.6	44.1	61.8	55.9	64.7	**79.4**
Enterobacter spp.	S	6.3	6.3	31.3	12.5	37.5	37.5	43.8	25.0	75.0	**81.3**	43.8	**93.8**	62.5	75.0	68.8	**93.8**	62.5	50.0	**87.5**	6.3
	I	00	00	00	00	00	00	00	6.3	00	00	00	00	12.5	00	25.0	00	18.8	00	00	12.5
	R	93.8	93.8	68.8	**87.5**	62.5	62.5	56.3	68.8	25.0	18.8	56.3	6.3	25.0	25.0	6.3	6.3	18.8	50.0	12.5	**81.3**

Bold numbers: Highest level of Resistance/Sensitivity.

most essential procedure to reduce bacterial load in open lower limb fractures [14]. The infection rate from the ward (third) samples was 45.1 %, where the post-debridement contamination was less (26.4%). Though, contamination played a significant role for ultimate wound infection [27], 73.6% of patients entered the ward with no contamination, but among them infection developed in 34.63% of cases. The organisms found in the third specimen culture were significantly different than the post debridement culture [28]. Furthermore, new organisms were identified from the third (ward) culture. This indicates hospital-acquired infection. A review article on hospital-acquired infection from Singapore has reported that nosocomial infections are a significant issue worldwide, ranging from 5-10 % in European countries to more than 40% in Asia [29]. Like previous works increasing age, smoking, multiple co-morbidities, application of external fixator or wound closure at 1st surgery, failure to cover the wound within 5 days, higher Gustilo grade were significant risk factors for ultimate wound infection but alcoholism was not a risk factor in our analysis [30,31]. Our country has an extremely low rate of alcohol consumption [32]. Furthermore, studies have found that drinking alcohol has little effect on wound infection [33,34]. Alcohol use disorder may, however, play a role, but none of our subjects had this illness [35].

The bacteriological study found Gram-negative organisms in all three cultures, namely Pseudomonas spp., *Escherichia coli*, Klebsiella spp., Acinetobacter spp., Enterobacter spp. and Proteus spp. Though Gram-positive *Staphylococcus aureus* was found significantly in the first culture, they contribute minimally (1.4%) to infect the fracture site. Omid Jamei and his colleagues (2017), in their study of orthopaedic infections, expressed anxiety that the number of orthopaedic infections due to Gram-negative pathogens might rise in the future, especially for Pseudomonas spp. and Enterobacter spp. [36]. In the present study, Pseudomonas was the highest infecting organism (51.1%). Another study on orthopaedic infectious carried out at a tertiary care teaching hospital from India also reported a similar type of spectrum of organisms, except Acinetobacter spp. replacing Citrobacter spp in our cases. But the contribution of Citrobacter spp. or Acinetobacter spp. as finally infecting organism was minimal (5—6%) in both cases. That study from India also reported Gram-positive *Staphylococcus aureus* (48.4%) as the primary infection organism [37]. At the same time, another related study from the same country two years apart showed that 76% of bacterial isolates were Gram-negative, which was consistent with our findings [38]. This illustrates the heterogeneity in bacterial diversity and the need for updated knowledge from time to time, even from the same geographical area.

In the present study, common Gram-negative and Gram-positive organisms were alarmingly multidrug-resistant. Pseudomonas spp and Klebsiella spp. were only sensitive to intravenous imipenem or meropenem, (in 50—69% cases). A study on 126 patients in China in the year 2016 reported that Gram-positive bacteria were susceptible to meropenem and imipenem, while sulbactam and ampicillin displayed little activity [8]. Less commonly used antibiotics; co-trimoxazole and chloramphenicol showed good sensitivity against *Staphylococcus aureus* (90%) and *E. coli* (69—75%), but these old antibiotics were only 12—20% active against Pseudomonas spp. cultured. Netilmicin was 100% sensitive for *Staphylococcus aureus*. All the used antibiotics were 80—100% resistant in the cases of Proteus spp. (except for intravenous imipenem or meropenem which worked well (80—94% sensitive) against this pathogen). A study from the African continent reported similar multidrug resistance of Gram-negative isolates [19]. The highest sensitivity for Acinetobacter spp. was 58.8% to netilmicin, while levofloxacin and amikacin showed good sensitivity (93.8%) against Enterobacter spp.

Our study had a number of limitations, one of which was that we did not follow any specific antibiotic or debridement guidelines in order to examine the existing practice at our institute. Furthermore, ours was a single-centered observational study, and being a cross-sectional study design; follow-up details were not available. However, according to literature, guidelines differ from place to place, particularly when antibiotics and their resistance pattern is a concern. Nevertheless, from the findings of the antibiograms and diversity in the bacterial number and spectrum from three successive cultures, it is clear that our orthopaedic wards might be the source of new infections. This work will establish baseline data for the future trial of various guidelines from other countries at our institute and to establish a local guideline for our orthopaedic surgeons.

Conclusion

This study found that surgical debridement was effective in reducing contamination from the open fracture wound, but hospital-acquired infection was common in orthopaedic admitted patients. Gram-negative pathogens were dominant in infecting open tibia fracture; namely, Pseudomonas spp., *Escherichia coli*, Klebsiella spp., Acinetobacter spp., Enterobacter spp. and Proteus spp. and the antibiograms showed an alarming pattern of multidrug resistance. This effort will help in performing future trials of other countries' guidelines at our institute.

Credit author statement

Md. Samiul Islam: Methodology, Review editing. Syed Shahidul Islam: Conceptualization, Supervision. Sultana Parvin: Software, Data analysis, Statistical analysis. Mushfique Manjur: Literature search, drafting the article. Muhammad Rafiqul Islam: Data acquisition. Rabin Chandra Halder: Data acquisition. Mohd. Sayedul Islam: Data acquisition. Syed Khaledur Rahman: Data acquisition. Mobinul Hoque:Data analysis, Statistical analysis. Md. Omar Faruque: Data acquisition. A K M Nazmul Haque: Literature search, drafting the article.

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Conflict of interest statement

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