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Incidence of Rod Shaped Gram-negative Hospital Acquired Bacterial Infection and Antibiotic Resistance: A Retrospective Based Population Study

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ABSTRACT

The gram-negative, especially rod shapes vary in the frequencies that they cause the most frequent types of hospital-acquired infection: pneumonia, surgical site infection, urinary tract infection, diabetic infection, and burn and bloodstream infection. The presented study determined the microbial spectrum and antimicrobial susceptibility of gram-negative bacteria isolated from various infection sites in hospitalized patients in Sulaimani city. This study included 735 patients who underwent surgical and wound treatment admitted to the hospital. A microbiological standard technique was used for Identification, isolation and antimicrobial susceptibility. The data in this job were scaled into excel sheets and transferred to SPSS (Statistical Package for Social Sciences) version 24.0 software. The significance of associations between variables and predictor done by Chi-square ($\chi 2$). P-value of <0.05 was regarded significant. Male was the most common cases 52.5%. Patients less than 18 years-old were the most frequently affected 54.3%. Gram negative infection is the most common and most serious complication of burn injuries and it is the majority suspected clinical finding nearly to 60%. The most common bacteria separated from wound and sepsis were Pseudomonas aeruginosa 91.91%. Individual pathogens' incidence differ significantly between location of infection p<0.001. Pseudomonas aeruginosa, only resistant stabled at the lowest rates for Colistine and Imepenim (0.1% and 7.8%) and

significant finding was confirmed p<0.001. But highly resistance to Ticarcillin/Clavulanic Acid, Cefepime, Tobramycin, and Gentamicin (85.6%, 80.8%, 79.3%, 79.2%)
In conclusion, these population-based study gram-negative infections predominate in burn wounds. Microbial resistance to the confirmed drugs leads to repeated antimicrobial treatment modifications and long-time of treatments.

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1. INTRODUCTION

Incidences of hospital acquired or nosocomial infection vary in frequencies and the most common causes include; pneumonia, surgical site infection, urinary and blood stream infection. During the past years, because of the change in health surveillance, infection control practices and manufacturing more antimicrobial by different company the bacterial resistance especially, gram-negative bacteria associated with hospital and nosocomial infection [1].

Origins of micro-organisms were found in the patient's itself, from outside in the surrounding, and from health staff or family. The maximal sort type of hospital acquired infection were bloodstream infections, pneumonia, urinary tract infection, skin including burn wound and incision infections. Pathogens such as gram-positive, gram-negative bacteria, fungal and viral and are the major causes of nosocomial infections. Common problem in the treatment of hospital acquired infections in patient wards in hospitals are increasing the frequency of antibiotic-resistant organisms, usually within a week of the injury [2, 3].

Burn injury is the most common types to likes to develop infection. The burn area with more pathogens increase the risk of drug resistance, illustrates the need for a drug policy by the hospitals for burn patients. The separated bacteria showed high resistances to antibiotics. The results show that antibiotics should be given logically in burn suited striped by the bacterial resistance manner [4].

Exposed open complicated wound site that containing diet tissue make burned patients more likely to be infected. In addition, a general condition of immunodepression is due to defect in the action of neutrophils and the immune system. In these situations, pathogens readily can grow and increase in number. Also, Burn patients need more care in intensive care units for long duration, may be intubated and vine section and urinary bladder catheterization; also these places the climate is very infected [5].The parentage of contamination is related to the extent of the open wound [6].

In general, screening activities were the 1st steps in developed control of infection, which helps in decreasing the emerging of infections and reducing costs. Systematic screening of infection in burn patients should be applied, systemically and clinically to evaluate incidence and rates the risks of infection. At least, systematic screening of patients includes data collection of burn wound, non-wound. Wound surveillance includes sign of healing (granulation), change to infection, and follow up of wound cultures. Non-wound surveillance consists of monitor respiratory, urinary and gastro intestinal tract [7, 8].

2. LITREATURE REVIEW

More of virulence factors plays important role in wound infections when single or several microorganisms which present in a wound or around the environment surrounding the wounds. The natural immune systems invade the microorganism in viable cell and tissues

provoke a series of localized or systematic human response. The progression of wounds to patients itself wound contamination, and likelihood of increasing injury infection. Incidence of surgical, burn and diabetic wound infection are recognized having microbial etiology especially gram-negative bacterial microorganisms [9].

Operational wound infection

The occurrence of operational wound infection is based on the microbial contamination susceptibility of wound, post-operative wound infection carried out by clean surgery 1 to 2% risks of infection and not cleaned of procedures are more significant to patients itself contamination previous study estimated 17% of infection [10]. Regarding to the different medical records in previous studies, the surgical wound infection rates are relatively low 2 to 6.8% [11, 12, 13], but in the last two previous studies the rate of surgical wound infection are increased 40 and 52.9% [14, 15].

Soft tissue infection

Microbial wound infection investigations cause cutaneous abscesses, necrotizing infection and traumatic wounds. Previous studies shown that cutaneous abscesses approximately 25 to 30% of infection [16, 17]. But other studies revealed that nearly 30 to 50 of cutaneous abscesses were infected [16, 18, 19].

Bite wound infection

Because of the complex nature of microorganisms in the oral of human and animals most of the pathogens harmful and harbor which leads to infection on the area of bites such as (*provotella, porphyromonas, bacteroides and peptostreptococcus species)* [20]. Previous study demonstrated that 10 to 50% of infection depends on the gravidity and location of bite, and more than 20% of bites caused by dog, while 30 to 50% of infected wound caused by cat bite [21].

Leg and foot lesion infections

Leg and foot lesion infection develop as a continued outcomes of skin pressure up to bone prominences, which cause skin erosion, ischemia to local tissues and necrosis. More than 25% of leg and foot ulcer infection caused by underlying osteomyelitis and bacteremia [22, 23]. Approximately, 36% of the total number of bacterial infection caused by gram-negative bacterial isolate from chronic ulcer of leg and foot [24], while low incidence of leg and foot ulcer infection isolates shown about 5% [23].

Burn wound infection

Regarding to the National Healthcare Safety Network (NHSN), hospital acquired infections are centralized or systemic status caused by adverse response to the presence of pathogens or its toxins. Hospital acquired infections develops during patient's admission to hospitalization. It causes a common problem in health care facilities, leads to long duration hospital stays, actual morbidity and mortality, and high costs. Usually $\geq$ 48 hours after admission bacterial will produce and can crow which lead to hospital-acquired infections [25, 26].

Big open wound sites that containing dead tissue makes burned patients more likely to get infection. Also, a general condition of immune depression from loss of functioning of neutrophils and the cellular and humeral immune cells lead to infection. In these conditions, pathogens can easily reproduce and grow wounds to high consistency. Patients by burn are need to remain for log periods intensive care units, during which they may be intubated with endotracheal and/or bladder catheterization which are the risk of contamination [27]. The degree or amount of contamination is depending to the opened wound size [28].

Provenances of pathogens are found in the patients itself, from surrounding, and from healthcare staff or families. The most common type of Hospital acquired infections in burned patients are circulatory infections, pneumonia, urinary tract infection, soft and operational areas infections. Gram-negative bacteria, gram-positive bacteria, yeast and viral pathogens are most common causes of burned hospital acquired infections. Antibiotic-resistant is the most common issue in the management of hospital acquired infections in hospital wards after a

week of the burn damage [29, 30].
Also, drug resistances increase the emerging of multi drug pathogens, this need to follow the drug guidance for burn patients. The isolated bacteria exhibited multiple resistances to antibiotics [31,32].
Infection essentially produced by Microorganisms which invasive burn wound such as gram-negative organisms like; (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Serratia marcescens, Enterobacter species, Proteus species, Acinetobacter species, and Bacteroides species*).
Thermal injury is the most major causes of emerging bacterial infection which the biggest problem especially, in developing countries. Regarding to data from different medical reports in many countries, the pathogens etiology of burn patients were reported by: *P. aeruginosa* (25-74%), *E. coli* (5-35%), *Enterococcus species* (9-14%), *S. aureus* (9-17%), *CoNS* (2-21%), *A. baumannii* (1-24%) [33]. The isolated infectious agents were*: Pseudomonas spp.* (36.84 %), *Acinetobacter species* (28.07 %), and *E. coli* (7.01%), *Enterobacter species* 5.26 %, *S. aureus* and *Candidia albicans* 3.50 % [34], however, *Pseudomonas spp.* Isolates were the commonest bacteria (20.4 %) followed by *MRSA* (8.1 %%), *Enterobacter species* (2.6 %), *MRCNS* (2.1%), *E. coli* (1.7 %) and *Acinetobacter species* (0.9 %) [35].

Antibiotic resistance

Antibiotic resistance common in hospital acquired infections. Gram-negative bacterial separation classified in to three or more first line classes depending to the multi-drug resistance of antimicrobials; beta lactams, aminoglycoside, and fluroquinolone in which they are resistance to ≥3 drugs (3^{rd}/4^{th} descent Cephalosporins, Piperacillin/tazobactam, Imipenem, Meropenem, Fluoroquinolones, and Aminoglycosides) [36].
A previous study in North Iran revealed that *Pseudomonas spp.* has less resistant to antibiotics like Amikacin (50%), Gentamicin (42.85%), Ciprofloxacin (94.4%), Carbenicillin (42.85%), Tobramycin (87.52%) and Ceftazidime (33.3%) [34].
But a study in Iraq revealed that *Pseudomonas spp.* is the commonest multi-drug resistance for Amoxicillin, Augmentin, Cefadroxil, and Cefotaxime 100%, followed by Klebsella species about 100% MDR for Amoxicillin, Augmentin, Cefadroxil, Cefotaxime, and Co-Trimoxazole. While, *staphylococcus. arueus* was less resistant to above antibacterial agents (39.7- 61.9%) [37].

3. METHODS AND MATERIALS

The presented study determined the microbial spectrum and antimicrobial susceptibility of rod shaped gram-negative bacteria separates from several infection areas in admitted patients to hospitals including operation room, patient wards and intensive care units in Sulaimani city. This study included 735 patients who underwent surgical and wound treatment admitted to the hospitals between January 2015 to end of December 2018.
To obtain the objectives of this fulfillment work, a structured data collection sheet about wound injury and infection patterns were used to estimate the specialty of patients and most of related factors to infections, microbial determination, and antibiotic sensitivity.
Consistency, separation and antimicrobial sensitivity checking of bacterial isolates were done by using standard techniques of microbial investigations.
The swabs of the wound were taken on admission. Almost, the swabs were taken before dressing and before drug administration wherever possible. Also, the swabs were at the times were clinical signs of skin infections appeared and cultures of urine cultures were taken per week for those with intubated urinary catheterization and on the onset signs and symptoms of urinary tract problem. Antibiotic sensitivity test was taken at the microbiological laboratory inside those hospitals.
The data in this job were scaled into excel sheets and transferred to SPSS (Statistical Package for Social Sciences) version 24.0 software. The significance of associations between variable

and predictors confirmed by Chi-square ($\chi 2$). P-value of <0.05 was regarded significant.

4. RESULTS

A total of 735 patients were studied, in which 386 (52.5%) patients were males and 349 (47.5%) were females. The mean age is 25.9 years (ranged from 10 month to 96 years-old). Patients less than 18 years of age reported more proportion of the admitted patients 399 (54.3%) followed by more than 40 years-old 184 (25%). Individual pathogens' incidence differ significantly between age groups p<0.001. (Table1).

Table 1: Patients characteristics in relation to individual pathogenic infection

Patients characteristics		No.	%	p-value
Gender	**Male**	386	52.5	1.45
	Female	349	47.5	
Age group	**<18**	399	54.3	0.001
	18-40	152	20.7	
	>40	184	25	

The study shown that burn open wounds were most frequently infected 437 (59.3) followed by Surgical closed wound, surgical opened wound and blood culture 192 (26.1%), 67 (9.1%) and 22 (3.0%), respectively. (Table 2)

Table 2: Distribution site of infection in the patient's body

Site of infection	No.	%
Bed Sore	6	.8
Blood culture	22	3.0
Burn open wound	436	59.3
Diabetic Wound	2	.3
Surgical open wound	67	9.1
Surgical closed wound	192	26.1
Urine culture	10	1.4
Total	735	100

The most common bacteria isolated from wound and sepsis were *pseudomonas species* especially*, pseudomonas aeruginosa* 455 (62.0%), followed by *providencia species* especially *Providencia stuartii* 52 (7.08%) and *Proteus species* especially*, Proteus rettgeri* 14 (1.91%). While *Aeromons salmonicida* were less isolated bacteria 4 (0.55%). (Table 3)

Table 3: Distribution of bacterial isolate

Bacterial isolate	No.	%
Achromonas xylosis	6	0.82
Aeromonas hydrophilia	11	1.50
Aeromons salmonicida	4	0.55
Burkholderia cepaciae	5	0.68
Pasteurella pneumotropica	5	0.68
Proteus mirabilis	9	1.23
Proteus rettgeri	14	1.91
Providencia rettgeri	18	2.45
Providencia stuartii	52	7.08
Pseudomonas aeruginosa	455	61.91
Pseudomonas fluorescens	50	6.81
Pseudomonas Fluorescens	44	5.99
Pseudomonas luteola	20	2.73
Pseudomonas putida	18	2.45
Pseudomonas stutzeri	5	0.68

Ralstonia pickettii	11	1.50
Stenotrophomonas maltophilia	8	1.09

The highest resistances were seen against for Ticracillin-Clavic Acid 619 (84.2%) followed by Tobramycin, Gentamicin, Cefepime and Ceftazidim 577 (78.5%), 576 (78.4%), 569 (77.4%), and 419 (57.0%) respectively. While most antibiotic active against (sensitive) seen for Colistine and Imipenem 730 (99.3%) and 607 (82.6%). (Table 4)

Table 4: Distribution of antibiotic sensitivity test.

Antibiotics	I No. (%)	R No. (%)	S No. (%)	Total No.
Ticarcillin/Clavulanic Acid	11 (1.5)	619 (84.2)	105 (14.3)	735
Cefipime	41 (5.6)	569 (77.4)	125 (17.0)	735
Imipenem	68 (9.3)	60 (8.2)	607 (82.6)	735
Meropenem	60 (8.2)	353 (48.0)	322 (43.8)	735
Ceftazidim	20 (2.7)	419 (57.0)	296 (40.3)	735
Amikacin	57 (7.8)	388 (52.8)	290 (39.5)	735
Gentamicin	12 (1.6)	576 (78.4)	147 (20.0)	735
Tobramycin	18 (2.4)	577 (78.5)	140 (19.0)	735
Ciprofloxacin	63 (8.6)	291 (39.6)	381 (51.8)	735
Colistine	0 (0.0)	5 (0.7)	730 (99.3)	735
I: intermediate, R: resistant, S: sensitive				

From the total of 455 *Pseudomonas aeruginosa* samples, the more common areas of infection founded were burned injury infection 256, followed by surgical closed wound and surgical open wound 109 and 32. But for the other site of infection the number were in different. (Table 5)

Table 5: Bacterial identification in relation to site of infection. ($p<0.001$)

Site of infection / Bacterial isolate	Bed Sore No.	Blood culture No.	Burn open wound No.	Diabetic Wound No.	Surgical open wound No.	Surgical closed wound No.	Urine culture No.	Total No.
Achromonas xylosis	1	0	3	0	0	2	0	6
Aeromonas hydrophilia	0	1	5	0	1	3	1	11
Aeromonsa salmonicida	0	0	1	0	0	3	0	4
Burkholderia cepaciae	0	0	1	0	0	4	0	5
Pasteurella pneumotropica	0	0	2	0	0	3	0	5
proteus mirabilis	0	1	4	0	0	4	0	9
Proteus rettgeri	0	1	7	0	1	5	0	14
Providencia rettgeri	0	0	10	0	2	6	0	18
Providentia stuartii	0	5	30	0	8	7	2	52
Pseudomonas aeruginosa	1	5	303	2	32	109	3	455
Pseudomonas fluorescens	2	2	20	0	12	12	2	50
Pseudomonas Fluorescens	1	3	16	0	8	14	2	44
Pseudomonas luteola	1	2	12	0	2	3	0	20
Pseudomonas putida	0	1	10	0	1	6	0	18
Pseudomonas stutzeri	0	0	2	0	0	3	0	5
Ralstonia pickettii	0	0	5	0	0	6	0	11
Stenotrophomonas maltophilia	0	2	4	0	0	2	0	8

Total	6	22	436	2	67	192	10	735

For *pseudomonas aeruginosa*, the resistances of antibiotics were seen against to Ticarcillin/Clavulanic Acid, Cefipime, Tobramycin and Gentamicin 85.6%, 80.8%, 79.3%, and 79.2% respectively. But most antibiotic were sensitive for *pseudomonas aeruginosa* and less resistance are Colistine and Imipenem 0.1% and 7.8%. In addition, Colistine seen the less or no resistance for all of the bacterial isolate ranged from 0.0% to 0.1%. (Table 6).

Table 6: Antibiotic resistance according to bacterial isolates. (%)

Site of infection / Bacterial isolate	**TCC %**	**CPM %**	**IMP %**	**MRP %**	**CL %**	**AK %**	**GEN %**	**TOB %**	**CIP %**	**CAZ %**
Achromonas xylosis	-	96.2	-	-	-	100.0	100.0	100.0	-	-
Aeromonas hydrophilia	-	26.9	-	-	-	-	-	-	-	-
Aeromonsa salmonicida	100.0	92.3	100.0	100.0	-	100.0	100.0	100.0	-	-
Burkholderia cepaciae	100.0	96.2	-	-	-	100.0	100.0	100.0	-	100.0
Pasteurella pneumotropica	100.0	57.7	100.0	100.0	-	100.0	100.0	100.0	100.0	100.0
proteus mirabilis	-	42.3	-	-	-	-	100.0	100.0	-	-
Proteus rettgeri	-	96.2	-	-	-	-	-	-	-	-
Providencia rettgeri	-	96.2	-	-	-	100.0	100.0	100.0	-	100.0
Providentia stuartii	-	96.2	-	-	-	33.3	33.3	33.3	33.3	-
Pseudomonas aeruginosa	85.6	80.8	7.8	49.1	0.1	52.8	79.2	79.3	40.2	57.6
Pseudomonas fluorescens	100.0	-	-	20.0	-	100.0	100.0	100.0	80.0	100.0
Pseudomonas Fluorescens	-	96.2	-	-	-	-	-	-	-	50.0
Pseudomonas luteola	50.0		100.0	100.0	-	-	100.0	100.0	100.0	100.0
Pseudomonas putida	-		-	-	-	100.0	100.0	100.0	-	-
Pseudomonas stutzeri	40.0		-	-	-	-	-	-	-	20.0
Ralstonia pickettii	100.0		-	-	-	-	-	-	-	100.0
Stenotrophomons maltophilia	50.0		100.0	100.0	-	100.0	100.0	100.0	-	-
p-value	0.000	0.000	0.001	0.5	0.000	0.3	0.01	0.02	0.05	0.000

TCC: Ticarcillin/Clavulanic Acid, CTX: Cefotaxime, CAZ: Ceftazidime, CPM: Cefipime, MRP: Meropenem, IMP: Imipenem, AK: Amikacin, GEN: Gentamicin, CIP: Ciprofloxacin, ATM: Aztreonam, TOB: Tobramycin, CL: Colistine.

5. DISCUSSION

It is confirmed that effective screening and soon detection of infections helps in better treatment of patients and decrease incidence infection, mortality average, duration of hospitalization and associated cost especially, in our community. We found that male was more affected than female and the mean age was 29.9 years-old, it is nearly consistent to the previous study by Ngai Kien Le et al. [38]. Nevertheless, other study have reported that female was more affected than male and mean age was higher compared to the current study[39].

There was a big difference between age groups, and we found that pediatric age were more likely to get infection. Also statistical significant was founded p=0.001. Similar result was found in the previous study revealed that pediatric age was the most frequent patient's affected [high prevalence]. But another study showed that old ages were more frequent patients to get infection [40]. Pediatric ages are susceptible to most infection because they didn't yet develop immunity. Also close proximity of high proportion numbers of infection and them susceptible host favors to transmit infection, and behavioral or emotional characteristics of them such as; inadequate hygiene, direct contact to ply objects, frequent mouthing of hand, incontinence and frequent contact with facilitate are risk for getting infection [41, 42, 43].

This study established that burn wound is the most frequent causing hospital acquired infection and with rod shaped gram-negative bacterial infection. This could be due to centralized or systemic status caused by adverse response to the presence of pathogens or its toxins. Hospital acquired infections develops during patient's admission to hospitalization. It causes a common problem in health care facilities, leads to long duration hospital stays, actual morbidity and mortality, and high costs. Usually $\geq$ 48 hours after admission bacterial will produce and can crow which lead to hospital-acquired infections [25, 26]. Previous studies agree with this finding [44, 45].

In the current study, the more common bacterial isolates from injury and sepsis were *pseudomonas species* especially, *pseudomonas aeruginosa*. Similar result was found in the previous study [38]. Also *pseudomonas aeruginosa* is founded more frequently among burn wound infected patients. Statically significant was found for these differences $p<0.001$.

Ticarcillin/Clavulanic Acid, Cefipime, Tobramycin and Gentamicin sowed the high resistance rate than the other antibiotics. But Colistin and Imipenem are with less resistance rate.

The antimicrobial drugs with antibiotic resistant proportion to *P. aeruginosa* more than 70% includes; Ticarcillin/Clavulanic Acid, Cefipime, Tobramycin and Gentamicin, while resistant rate for Colostine and Imipenem was lower than 10%. This result is agree to the previous study which is conducted by Souli, M., I. et al in Europe [46]. Another study by Jafar, E., M.R. Shakibaie, and L. Poormasoomi showed that *P. aeruginosa* has another resistant proportion because of the plasmid genes encoded in different, more stability, and low healing efficiency and easy transmission through connecting to the other hospital acquired microbes [47].

Moreover, Resistances rate are less for *Aeromonsa salmonicida, Burkholderia cepaciae, Pasteurella pneumotropica, Pseudomonas luteola, Pseudomonas fluorescens, and Stenotrophomonas maltophilia relaitivily high for most of the antibiotics, while Aeromonas hydrophilia, Proteus rettgeri, Pseudomonas Fluorescens, and Pseudomonas stutzeri, and Ralstonia pickettii.* This could be due to the frequency of isolates of those bacteria. Repeated using of mentioned antibacterial may be common factors leads to raises of resistant proportions for the most of them and cause impairment drugs action.

6. CONCLUSION

Challenges for hospitals infection and control is to prevent passed community acquired infection especially, inside the hospitals and other health care unite or environment. The study showed that rod shape gram-negative bacteria especially, *Pseudomonas aeruginosa* was high risk for hospital acquired infection. Burn wound was the most dangerous site in human body like to get infection. Antibacterial management modifications were more repeated and the treatment time was longer than observed because of the microbial resistance to the confirmed drugs. Also most of those antibiotics have no effect to reducing the infection.

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