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Minimum Inhibitory Concentration of Tigecycline on *Acinetobacter* in Burn Patients

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ABSTRACT

Background: Burn infection is a major cause of morbidity and mortality in spite of significant improvements in burn care and treatment. *Pseudomonas aeruginosa, Acinetobacter spp., Staphylococcus aureus* etc. are the commonest isolates in which rapid development of resistance to multiple drugs limits the therapeutic options for infections by *Acinetobacter* species. Hence, this study was done to find the occurrence of *Acinetobacter* and to determine the minimum inhibitory concentration of tigecycline against *Acinetobacter* isolates.

Methods: This cross-sectional study was conducted in Phect-Nepal Hospital, Kirtipur, Nepal from September to December 2018. Total 205 samples were included for the isolation and identification of Acinetobacter and further minimum inhibitory concentration of isolates were done following the standard laboratory protocol. Collected data were analyzed by SPSS version 23.0.

Results: Among 155 culture positive samples, 27 isolates were *Acinetobacter* spp. Antimicrobial Susceptibility Test revealed that 24 isolates were resistant to ceftriaxone and ceftazidime, but all isolates were susceptible to polymyxin B. For tigecycline, 19 isolates were resistant through dis diffusion test while 20 isolates cross the Minimum Inhibitory Concentration value from E test. The reliability of the E-test and disc diffusion was 0.920, which represent strong agreement between E- test and dis diffusion test.

Conclusions: Tigecycline resistance is presenting as serious problem to the management of infection caused by *Acinetobacter* species.Therefore, minimum inhibitory concentration for the detection of resistance should be included in routine laboratory diagnosis.

Keywords: *Acinetobacter* spp.; AST; burn; MIC; tigecycline

INTRODUCTION

Skin, covering our whole body, is one of the largest organ and an essential component of the body.[1] Burn is one of the most common and devastating form of trauma caused by external heat sources, such as radiation, electrical and chemical.[2,3] The most frequent isolated from burn patients were *Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter* spp in which *Acinetobacter* emerged as notorious organism to develop antibiotics resistance rapidly in response to challenge with new antibiotics.[4, 5]

Burn infection is one of the major cause of morbidity and mortality, accounting for quarter of nosocomial infections though various measures has been applied for infection control and burn wound management.[2, 6] Increase in the resistance of the *Acinetobacter* against Tigecycline is leading problem in course of treatment.[7, 8] Thus, this study reveals the current scenario of minimum inhibitory concentration (MIC) of the tigecycline on *Acinetobacter* spp. from burn patients.

METHODS

The study was hospital based cross sectional study conducted in the Kirtipur Hospital, Phect- NEPAL from September to December 2018. Ethical approval was obtained from Institutional Review Committee of Phect- NEPAL. The study population was the patients with burn infection of all age group and sex visited or admitted during the periods of study and sample was collected after written or verbal consent of care taker of patient. The samples (Wound Swabs, Tissues, central

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venous pressure (CVP) Tips, Foley's Tips, Pus, Catheter Tips, femoral tips, Drain) were collected by well-trained hospital staff or researcher after 24 hours. of burn injury. Any leaked or mislabeled or sample collected by nonclinical staff were not included in this study. All the samples obtained within three months of period were included in this study.

The collected sample was processed for the microbiological diagnosis. After Gram stain (Hi Media, India) of sample, it was directly inoculated on freshly prepared media Mac-Conkey (Hi Media, India) and Blood agar (Hi Media, India), and incubated aerobically at 37°C for 24 hours. The plates were observed for bacterial growth after incubation, if any growth were seen then the identification of the isolates was done by following standard laboratory method.[9] Antimicrobial Susceptibility Test (AST) was done by using Kirby Bauer's dis diffusion method described in CLSI, 2018 and MIC was determined by Epsilometer test (E-test).[10]

Specifically, the Food and Drug Administration (FDA) approved MIC break- points for susceptibility and resistance are ≤2 mg/L and ≥8 mg/L, respectively and the zone diameter of tigecycline for *Acinetobacter* spp. including multidrug resistant strains is ≥ 19 / ≤14 mm defining the susceptibility/resistance.[11-14]

All findings were entered in Microsoft excel version 2013 data sheet and IBM Statistical Package for the Social Sciences (SPSS) 23.0 on completion of the study. Data were recorded manually maintaining confidentiality.

RESULTS

All 205 patients were grouped in their respective age group with class interval of 10. The highest number of patients was observed in class 20-30; 38 males and 15 females while the least number of patients were found to the class 80-90 including only 2 females. No patients were reported in the age between 60-70. Rest data were shown in Figure 1.

Among 205 samples, 36.09% were injured from electric burn and only 2.43% were reported with chemical burn (acid burn) injury (Table 1).

Table 1. Number of patients affected by different types of burn.

S.N.	Types of burns	Number of Patients (%)
1	Electric burn	74 (36.09)
2	Scald burn	57 (27.08)
3	Flame burn	37 (18.04)
4	Chemical burn	5 (2.43)
5	Others	32 (15.60)
Total		205

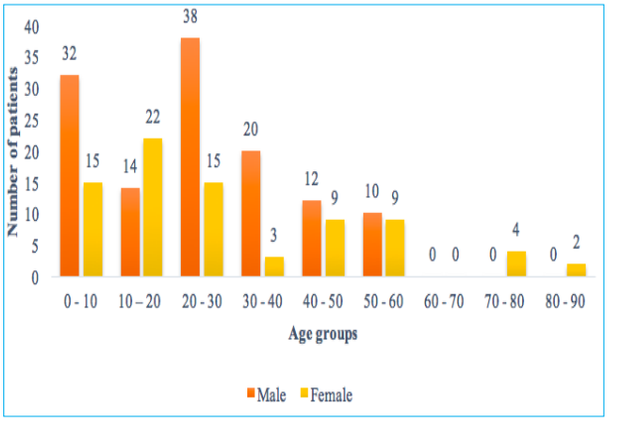

Figure 1. Age and sex wise distribution of patients.

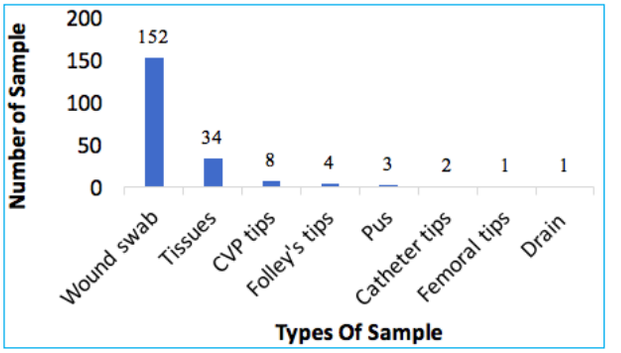

Figure 2. Types of samples.

Out of 205 samples, culture positive was found in 155 samples in which 27 samples showed growth of *Acinetobacter* spp. The most prominent *Acinetobacter* spp was Acinetobacter Calcoaceticus Baumanii Complex (ACBC).

In comparison to female (26%), male (74%) was mostly infected by *Acinetobacter* spp. among all the culture positive samples.

Different antibiotics were used to test the antibiogram of *Acinetobacter* spp. (Table 2). The susceptibility patterns were interpreted based on CLSI guidelines 2018. Except Polymyxin B, all classes of antibiotics were found resistant against *Acinetobacter* spp. The AST pattern revealed that ceftriaxone and Ceftazidime (88.89%) were highly resistant antibiotics followed by ciprofloxacin, doxycycline and meropenem each with 85.18%. Polymyxin B was 100% susceptible against Acinetobacter spp. (Table 2).

Table 2. Antimicrobial susceptibility pattern of Acinetobacter spp.

S.N.	Antibiotics	Sensitive	Resistance	Total
1	Amikacin	4 (14.82%)	23 (85.18%)	27
2	Cefepime	5 (18.52%)	22 (81.48%)	27
3	Ceftazidime	3 (11.11%)	24 (88.89%)	27
4	Ceftriaxone	3 (11.11%)	24 (88.89%)	27
5	Ciprofloxacin	4 (14.82%)	23 (85.18%)	27
6	Cotrimoxazole	5 (18.52%)	22 (81.48%)	27
7	Doxycycline	4 (14.82%)	23 (85.18%)	27
8	Gentamicin	5 (18.52%)	22 (81.48%)	27
9	Imipenem	5 (18.52%)	22 (81.48%)	27
10	Levofloxacin	5 (18.52%)	22 (81.48%)	27
11	Meropenem	4 (14.82%)	23 (85.18%)	27
12	Piperacilline/ tazobactum	5 (18.52%)	22 (81.48%)	27
13	Polymyxin B	27 (100%)	0	27

Table 3. Resistance pattern of tigecycline.

E- test			Disc diffusion		
Sensitive	Intermediate	Resistance	Sensitive	Intermediate	Resistance
6 (22.22%)	1 (3.70%)	20 (74.07%)	4 (14.81%)	4 (14.81%)	19 (70.37%)

Comparing between E-test and dis diffusion test, 74.07% (27) isolates were found resistant in E test than slightly decrease percent (70.37%) in dis diffusion test (Table 3).

The reliability analysis (alpha coefficient or Cronbach's Alpha) value between E test and dis diffusion test showing 0.92 further indicate that both tests have relatively high consistency.

DISCUSSION

Out of 205 sample, the age and sex distribution of total patients depicts the highest number of patients in age group of 20 – 30 with 38 males and 15 females. Incidence of burn was more common in this age group since they are the most active group and mostly involved in outdoor and indoor activities. Our study agrees with a similar study carried out by Chalise et al., 2008, Datta et al., 2016. [4, 6]

36.09% patients were mostly affected by electric burn as compared to other types of burn. This might be due to the unmanaged electric wires and unaware of the proper handling of the electric equipment. Moreover, the lowest number of burns was seen due to chemical i.e. acid burn, which is mostly because of the criminal activities like acid attack.

Out of 205 samples processed, 155 showed bacterial growth with single and multiple isolates among which only 27 *Acinetobacter* spp were identified, 74% in male and 26% in female samples. The relative higher cases in male may be due to their greater participation in outdoor activity. However, our findings are in contrast to a previous study by Rajbahak et al., 2014.[3]

The study was conducted with the purpose of characterizing Acinetobacter from samples obtained from the patients. In our study, the total number of Acinetobacter isolated was 27 which comprises mostly male than the female. Acinetobacter calcoaceticus baumanii complex (ACBC) was frequently isolated among *Acinetobacter* spp. ACBC acts as nosocomial pathogens that engraved its function in hospital and inpatients are prone to ACBC infections since they are either immuno-compromised, due to the treatment they are receiving, or they have undergone some invasive procedure. Because of this, inpatients are at the highest risk of ACBC infection.[3, 15]

The challenge in treating Acinetobacter infection is primarily attributed to its high intrinsic tolerance to most antibiotics.[16] Antibiotic resistance profile of Acinetobacter isolates was determined using 14 antibiotics, and this revealed that the isolates were most commonly resistant to ceftriaxone and ceftazidime each with 88.89%, followed by ciprofloxacin, amikacin, doxycycline and meropenem each with 85.18%. Likewise, levofloxacin, gentamicin, cefepime, cotrimoxazole, piperacillin/tazobactum, imipenem each with 81.48% were resistance. However, all the isolates were

susceptible to polymyxin B. In context of Nepal, *Acinetobacter* spp. was resistant to cotrimoxazole (5.3%) whereas it was more sensitive to amikacin (71.1%).[3] In comparison to our study the research conducted by Mindolli and Salmani, 2014, showed the least resistant strains to different antibiotics such as meropenem (9.5%), piperacillin tazobactum (9.5%), amikacin (37%), ceftazidime (38.5%), gentamicin (47.5%)[17] whereas in Zampar et al., 2017 strains were highly resistant to carbapenem (92%), fluoroquinolones (80%), amikacin (78%) and piperacillin/ tazobactum (51%).[18]

Determination of MIC by E-test showed that 74.07 % (20), 22.22% (6) and 3.70% (1) isolates were resistance, sensitive and intermediate to tigecycline respectively. The lowest MIC point of tigecycline was 0.5 µg/ml and the highest was 8µg/ml. According to the study of Navon-Venezia, Leavitt and Carmeli, 2007, MIC testing of tigecycline using E - test showed 66% of the MDR Acinetobacter spp. were resistant, 12% were intermediate and only 22% were susceptible.[19]

In the study performed by Shoja et al., 2017, MIC of Tigecycline showed that 55% of the isolates were sensitive, 10% intermediate, and 35% were resistant.[20]

CONCLUSIONS

Tigecycline resistance is presenting as serious problem to the management of infection caused by Acinetobacter as its prevalence is increasing. Acinetobacter being ESKAPE pathogen showed its clinical importance in antibiotic resistant trend. Having MIC test clearly showed how gradual changes in the minimum inhibition concentration of tigecycline in case of monotherapy. Therefore, MIC test should be included in routine laboratory diagnosis.

Now, with the increase in numbers of cases and increased resistance to antibiotics, it is more important to investigate and improve the prevention and treatment of this life-threatening organism.

CONFLICT OF INTEREST

None

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