Detection of MRSA and ESBL Producing Bacteria with Their Antimicrobial Resistance Pattern Isolated from Infected Burn Wounds in Rajshahi Medical College Hospital.

Md. Ahsanul Haque[a], Md. Shah Alam[b], Farjana Kabir[c], Rozina Aktar Zahan[d], Md. Mottalib Hossain Khan[e], Md. Sirazum Munir[f].

[a]Medical Officer, Department of Microbiology, Rajshahi Medical College, Rajshahi, Bangladesh.

[b]Professor and Head, Department of Microbiology, Barind Medical College, Rajshahi, Bangladesh

[c]Assistant professor, Department of Physiology, Pabna Medical College, Pabna,Bangladesh.

[d]Assistant Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi, Bangladesh.

[e]Assistant professor, Department of Microbiology, Pabna Medical College, Pabna, Bangladesh.

[f]Lecturer, Department of Virology, Rajshahi Medical College, Rajshahi, Bangladesh.

Correspondence to : M A Haque
ahsanulhaque19052012@gmail.com

Cite this as: BMCJ 2022; 8(2): Page 16-24

Received: 11 March 2022
Accepted: 4 May 2022

Abstract

Background: Antibiotic resistance caused by the *MRSA* and *ESBL* producing drug resistant bacteria is a worldwide problem. World Health Organization (WHO) described antimicrobial resistant microorganism as "nightmare bacteria" that "pose a catastrophic threat" to people in every country in the world. **Objectives:** To detect antimicrobial resistance pattern of *MRSA* and *ESBL* producing bacteria isolated from infected burn wound patients in Rajshahi region. **Methods:** This descriptive type of study was carried out in Burn and Plastic Surgery unit of Rajshahi Medical College Hospital, Rajshahi, Bangladesh. A total of 250 burn wound swabs collected from the admitted patients were constitute the sample size. The collected specimens were inoculated in blood agar, nutrient agar and MacConkey's agar media and incubated aerobically at 37^0 C for 24 hours. Susceptibility tests of the bacterial isolates were done by using the modified Kirby Bauer disk diffusion method on Mueller Hinton agar media. *MRS* Aproducing bacteria were identified by Cefoxitin disk diffusion test and *ESBL* producing bacteria were identified by disk diffusion test. **Results:** Out of total 250 samples, culture yielded growth were 213 (85.2%) and total 231 bacteria were identified. Among them 136 (58.8%) isolates were gram negative and 95 (41.2%) isolates were gram positive. Male Female ratio among the growth positive patients was 1:1.4. S. aureus was the predominant organism, 71 (30.8%) followed by *P.aeruginosa* 67 (29%), *E.coli* 43(18.7%) and *Klebsiella spp.*16 (6.9%). Among 71 isolated S. aureus, 33(46.5%) were identified as *MRSA.* Among 136 isolated gram negative bacteria, 64(47.1%) were phenotypically confirmed as *ESBL.* **Conclusion:** In Bangladesh, *MRSA* and *ESBL* producing strains of bacterial infections are now challenging in management. It is necessary to perform sensitivity test prior to start antimicrobial therapy for proper management. Routine screening test for *MRSA* and *ESBL* should be practiced to detect the carriers and treat them adequately and also reduce and control the spread of antimicrobial resistance.

Key words: burn wound infection, antimicrobial susceptibility pattern, *MRSA, ESBL.*

Introduction

Infection remains the most common cause of morbidity and mortality in burn patients. The diagnosis and management of burn wound infection remains challenging due to the many physiologic features unique to burn injury. A variety of factors increase the risk of developing burn wound infection, and individuals who sustain a severe burn have a particularly high risk for burn wound sepsis. The most common organisms are Staphylococcus and Pseudomonas; however, the epidemiology of burn wound infections has changed with time and also depends on geography. It is imperative to be aware of the flora and susceptibility of organisms in each burn unit to be able to treat burn wound infections effectively.[1]

Methicillin resistant Staphylococcus aureus, a strain of S.sureus resistant to methicillin, such strains also are resistant to oxacillin and nafcillin, cephalosporins, and imipenem. MRSA is the most prevalent pathogen isolated from hospitalized patients and the second most common from patients in outpatient settings. The gradual increase in the prevalence of MRSA is already evident from many reports.[2]

In a study of Bangladesh by Tasnim A *et al.* (2015)[3] showed *MRSA* producing *Staphylococcus aureus* were 33.33%. Another study in Pakistan by Junaid K *et al.* (2019)[4] where they found *MRSA* producing *Staphylococcus aureus* were 43.0%. In India, a study done by Mir M *et al.* (2012)[5] showed *MRSA* producing *Staphylococcus aureus* were 30.5%. Another study in India by Perween N *et al.* (2015)[6] where they found MRSA producing Staphylococcus aureus were 56.7%.

*ESBL*s are plasmid mediated β-lactamase capable of conferring bacterial resistance to the penicillin's, first, second and third generation cephalosporins and monobactems (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics and which are inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, tazobactam.[7] The threat of *ESBL* producing gram negative bacteria on the overall health sector has led to its recognition as one of the most deadliest bacteria in the world. World health organization has published the list of bacteria in 2017 which are causing serious impact on antimicrobial treatment due to their multidrug resistance. *ESBL* producing bacteria fall among the most critical group.[8]

In a study at Dhaka, Bangladesh by Rahman M *et al.* (2018)[9] showed ESBL producing bacteria were 31.4%. Another study in Bangladesh by Islam MS *et al.* (2012)[10] where they found *ESBL* producing bacteria were 22.1%. In India, a study done by Rathod VS *et al.* (2017)[11] showed ESBL producing bacteria were 61.5%. Another study in India by Rani VS *et al.* (2016)[12] where they found ESBL producing bacteria were 37.2%.

Regarding the antimicrobial resistance rates of ESBL producing gram negative bacteria in Bangladesh to third generation cephalosporins 80%-100%, to fluroquinolones, aminoglycosides, monobactum 60%-80% , to carbapenem 10%-30%.[13]

The antimicrobial resistance rates of methicillin resistance *Staphylococcus aureus* in Bangladesh to penicillin 100%, third generation cephalosporins 80%-90%, to fluroquinolones, aminoglycosides, macrolids 60%-80%, to vancomycin and carbapenem 0%- 30%.[14]

Methods

This cross sectional descriptive type of study was carried out among the patients admitted in Burn and Plastic Surgery unit of Rajshahi Medical College Hospital during the period of July 2017 to June 2018. A total of 250 burn wound swabs collected from the admitted patients were constitute the sample size. Ethical clearance for the study was taken from the Ethical Review Board and concerned authority, Rajshahi Medical College & Hospital. Antimicrobial susceptibility of the bacteria isolated from burn wound swab were analysed. Aerobic culture and sensitivity tests were done in the Microbiology department of RMC. All the specimens were inoculated in blood agar, nutrient agar and Mac Conkey's agar media and incubated aerobically at 37^0 C overnight. If culture plates showed the growth of bacteria then it was identified by their colony morphology, pigment production, haemolysis on blood agar plate, motility test, Gram staining and relevant biochemical tests. The identified bacteria were sub cultured and processed for drug sensitivity test and preserved for further use. Susceptibility tests of the bacterial isolates with different antimicrobials were done by using the modified Kirby Bauer disk diffusion method on Mueller Hinton agar media by commercially available antimicrobial disks.[15]

Detection of Methicillin resistant Staphylococcus aureus (MRSA)

Cefoxitin disk diffusion method :

All *Staphylococcus aureus* isolates were screened for methicillin resistance by using cefoxitin disk (30 µg).The inoculum size was adjusted with 0.5 McFarland's standard and incubating a lawn on Mueller Hinton agar at 35^0C for 24 hours with a cefoxitin disk (30 µg). According to the Clinical and Laboratory Standards Institute (CLSI) - 2015, a zone of growth inhibition around the cefoxitin disk of >22 mm ruled out *MRSA;* a zone size ≤ 21 mm indicated that the mecA gene is present and the isolate was reported as *MRSA.* Cefoxitin was used in place of oxacillin to detect *MRSA* as it is better inducer of the mecA gene, and test using cefoxitin give more reproducible and accurate results than tests with oxacillin.[15]

Detection of ESBL producing Enterobacteriaceae

- Screening
- Phenotypic confirmation

Screening for ESBL :

Screening for *ESBL* producing gram negative bacteria was carried out during AST by modified Kirby Bauer disk diffusion method. Cefotaxime, ceftazidime and ceftriaxone either alone or in combination when showed the desired zone of inhibition, indicated the presence of *ESBL* producing gram negative bacteria.[15]

According to CLSI guide line 2017 (Disk diffusion method):

Screening Antibiotics	Zone of inhibition
Ceftazidime (30 µg) Or	≤22 mm
Cefotaxime (30 µg) Or	≤27 mm
Ceftriaxone (30 µg)	≤25 mm

The use of more than one antimicrobial agent improves the sensitivity of ESBL detection.

Phenotypic confirmation by disc diffusion test (PCDDT):

After inoculation of a Mueller Hinton plate with the test organism, Ceftazidime 30 µg + Ceftazidime/Clavulanate 30/10

µg and Cefotaxime 30 µg + Cefotaxime/-Clavulanate 30/10 µg these four disc were placed 20 mm apart. The plate incubated at 35^0C for 18-20 hours. A ≥5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone phenotypically confirmed ESBL.[15]

Results:

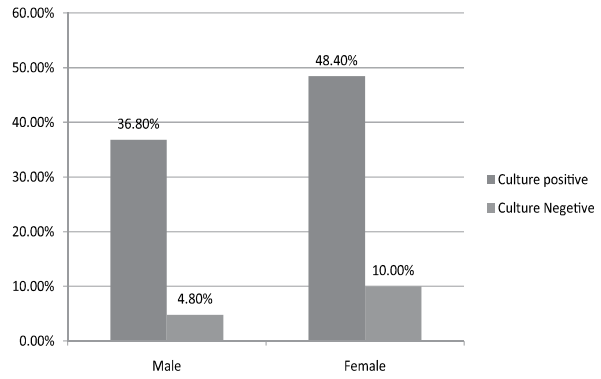

Figure I : Sex distribution of culture positive cases (n=250)

The female cases were 146 among them culture yielded growth were 121 (48.4%) cases and culture negative were 25 (10%). The male cases were 104, 92 (36.8%) cases were culture positive and 12 (4.8%) were culture negative (Figure I).

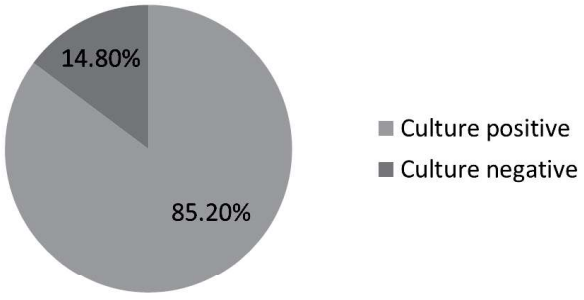

Figure II: Distribution of culture positive and negative cases (n=250).

Out of 250 samples, 213(85.2%) samples were culture positive while 37(14.8%) samples were culture negative (Figure II).

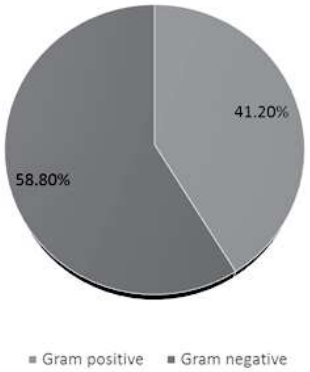

Figure III: Distribution of gram positive and gram negative bacteria (n=250)

A total of 231 isolates, Gram negative bacteria were 136(58.8%) and gram positive bacteria were 95(41.2%)(Figure III).

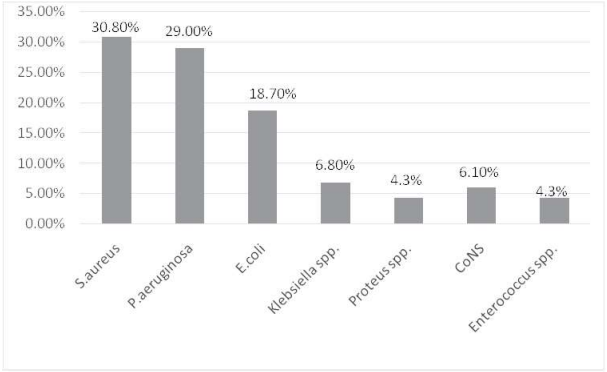

Figure IV: Pattern of bacteria isolated from burn wound samples (n=250).

Out of 250 samples, total 231 bacteria were identified. Among them S.aureus was 71(30.8%) followed by *P.aeruginosa* was 67(29%), *E. coli* was 43(18.7%) and *Klebsiella spp.* was 16 (6.9%) (Figure IV).

Table I: Distribution of ESBLand MRSA producing bacteria.

Isolates	Total No. of org. Tested	No. of positive org. confirmed by phenotypic method (%)
ESBL producinggram negative bacteria	136	64(47.1%)
Methicillin resistant *S.aureus*	71	33(46.5%)

Among the 71 isolated S. aureus, 33(46.5%) were identified as MRSA by cefoxitin disk diffusion test. Among 136 isolated gram negative bacteria, 64(47.1%) were phenotypically confirmed as ESBL producer by disk diffusion method (Table I).

Table II: Antimicrobial resistance pattern of MRSA producing S. aureus.(n=33).

Antimicrobial agents	*MRSA*resistance (%)
Imipenem	07(21.0)
Azithromycin	15(45.0)
Flucloxacillin	33(100.0)
Amikacin	22(67.0)
Ciprofloxacin	25(75.0)
Ceftriaxone	31(94.0)
Vancomycin	03(9.0)
Amoxiclav	24(72.0)
Cefuroxime	29(88.0)
Linezolid	05(15.0)
Cotrimoxazole	30(91.0)
Cefixime	31(94.0)
Doxycycline	21(63.0)

All of the MRSA was 100.0% resistant against flucloxacillin, ceftriaxone and cefixime was 94.0%, cotrimoxazole was 91.0%, cefuroxime was 88.0%, ciprofloxacin was 75.0% and amoxiclav was 72.0% resistant. Vancomycin, Linezolidand imipenem showed lower resistance; i.e., 9.0%, 15.0% and 21.0% against MRSA respectively (Table II).

Table III: Antimicrobial resistance pattern among ESBL producing gram negative bacteria (n=64).

Antimicrobial agents	*ESBL*resistance (%)
Imipenem	05(8.0)
Ciprofloxacin	28(44.0)
Ceftriaxone	54(84.0)
Ceftazidime	52(81.0)
Cefuroxime	56(88.0)
Azithromycin	40(62.0)
Aztreonam	34(53.0)
Amikacin	28(44.0)
Piperacillin/tazobactam	17(26.0)
Colistin	03(5.0)
Cefepime	15(24.0)
Doxycycline	32(50.0)
Cefixime	60(94.0)

Table-III shows the antimicrobial resistance pattern of the ESBL producing gram negative bacteria. All the ESBL producers were 80.0%-90.0% resistant against ceftriaxone, cefixime and ceftazidime. Colistin, imipenem and cefepime showed lower resistance; i.e.,5%, 8% and 24% against ESBL respectively.

Discussion

Out of 250 burn wound swabs obtained in the Microbiology laboratory from Burn and Plastic surgery unit of RMCH, Rajshahi for aerobic culture and sensitivity, 85.2% yielded positive culture whereas 14.8% yielded no growth. This findings in this study was nearly similar with the study of Anika *et al.* (2020)[16] and Rajbahak *et al.* (2014)[17] but dissimilar with the study of Jobayerh *et al.* (2021)[18] and Rani *et al.* (2016)[12].

The reason for this high occurrence of culture positivity may be due to the fact that most of the study population were belonged to lower middle and lower socioeconomic group with poor knowledge about personal hygiene, poor sanitation system in hospital, overcrowding of patients in hospital contribute to high rate of cross infection, inadequate measures for prevention of the spread of resistant pathogen in hospital environment.

The findings of the present study suggest, the burn wound infection rate was higher in the females than males. It was nearly similar with the studies of Anika *et al.* (2020)[16] and Sanjana *et al.* (2018)[19] but dissimilar with the study of Islam *et al.* (2013)[20] and Khurram *et al.*(2018)[21]. This higher infection cases in female patients may be due to the presence of poor nutrition, co-morbidity, malignancy, immunosuppression and hematological disorders.

Out of a total 250 samples, Gram negative bacteria were accounted for higher isolation rate than gram positive bacteria. These were nearly similar with the studies of Kaniz *et al.* (2021)[22] and Rani *et al.* (2016)[12] but nearly dissimilar with the study of Jobayer *et al.* (2021)[18] and Rathod *et al.* (2017)[11].

S.aureus were the most frequent isolates, 71(30.8%). It was similar with the study of Kaniz *et al.* (2021)[22] and Sanjana *et al.* (2018)[19] but dissimilar with Jobayer *et al.* (2021)[18] and Rani *et al.* (2016)[12]. The high prevalence of *S. aureus* infection may be because it is an endogenous source of infection and contamination of surgical instruments. With the disruption of natural skin barrier S.aureus, which is a common bacterium on surfaces, easily find their way into wounds.

A total of 231 bacterial isolates, 67(29.0%) were P.aeruginosa. Among isolated gram negative bacteria, It was the most common. This current study finding was similar with the studies of Chaudhary *et al.* (2019)[23] and Khurram *et al.* (2018)[21]. But it was dissimilar with the study of Jobayer *et al.* (2021)[18] and Rani *et al.* (2016)[12].

The present study noted that 47.1% of gram negative bacteria were *ESBL* producing strains. This was nearly similar with the study of Islam *et al.* (2012)[10] and Bandekar et al. (2011)[24] But different findings were reported by Rahman *et al.* (2018)[9] and Rathod *et al.* (2017).[11] This difference may be due to the fact that it is difficult to detect *ESBL* producers and its distribution varies between various geographical locations and hospitals.

Methicillin resistant *S. aureus (MRSA)* is another therapeutic challenge like *ESBL* producing bacteria. In this study, out of 71 S.aureus, 33(46.5%) isolates were *MRSA*. This was similar with the study of Shariful *et al.* (2013)[20] and Perween *et al.* (2015).[6] But dissimilar with the study of Anika *et al.* (2020)[16] and Sanjana *et al.* (2018).[19] This difference may be due to *MRSA* infection is variable from different hospitals, geographical locations and countries depending on antibiotic policy.

The isolated *MRSA* strains in this study were highly resistant to ceftriaxone, cefixime, ceftazidime, cefuroxime and flucloxacillin. But relatively lower resistance was observed against vancomycin, linezolid and imipenem. This study was nearly similar with Jobayer *et al.* (2021)[18] and Khurram *et al.* (2018)[21].

In this study all the ESBL strains of gram negative bacteria were highly resistant to

ceftriaxone, cefuroxime, cefixime and `ceftazidime. Colistin, imipenem and cefepime were effective against ESBL strains. This study were nearly similar with Jobayerh *et al.* (2017)[26] and Keerthi *et al.* (2017).[27] This variations may be due to differences in local conditions, prevention protocols, antibiotic policy as well as duration of study, variation in host and immune status of the host.

References

1. Sharma L, Srivastava H, Pipal DK, Dhawan R, Purohi PM, Bhargava A. Bacteriological profile of burn patients and Antimicrobial susceptibility pattern of burn wound isolates. International surgery journal 2017; 4(3):1019-23.
2. Haq J A, Rahman MM, Asna AH,et al. Methicillin-resistant Staphylococcus aureus in Bangladesh - A multicentre study. International Journal of Antimicrobial Agents 2005; 25(3):276-7.
3. Tasnim A, Shamsuzzaman AK, Ferdose J, Khan FA, Nahreen R. Current trend of aerobic bacteria andantimicrobial susceptibility pattern in burn wound infection of a tertiary care hospital, Rajshahi. Mymensingh Med J. 2017; 31(2):431-6.
4. Junaid K, Mustafa A , Arshad S , Farraj DA , Younas S , Ejaz H. Burn wound infections: a serious threat of multidrug-resistant Staphylococcus aureus. P J M H S. 2019; 13(3): 804-7.
5. . Mir M, Anjum S, Mir R, Sheikh G, Mir M, Reshi F. Prevalence of Various Bacteria And Their Antibiotic Sensitivity Pattern In Burn Unit Of Government Medical College And Hospital Srinagar. The Internet Journal of Microbiology. 2012; 10(1):1-6.
6. Perween N , Krishanprakash S , Bharara T. Bacteriological Profile of Burn Wound Infection in a Tertiary Care Hospital in North India with Special Reference to Methicillin Resistant Staphylococcus aureus. International Multispecialty Journal of Health 2015;8(1):6-11.
7. Bush K, Jacoby GA.Updated functional classification of beta lactamases. Antimicrob Agents Chemother 2010; 54(3): 969-76.
8. World Health Organization.Antimicrobial resistance. 2017. www.who.int/antimicrobial-resistance/en.
9. Rahman M, Sultana H, Mosawuir MA, Akhter L, Yusuf MA. Status of Extended Spectrum Beta-Lactamase (ESBL) Producing Bacteria Isolated from Surgical and Burn Wound at Tertiary Care Hospital in Dhaka City. Bangladesh J Infect Dis. 2018;5(1):21-6.
10. Islam MS, Yusuf MA, Chowdhury Ma, Hossain A. ESBL producing gram negative aerobic bacteria isolated from burn wound infection with their antibiogram in Dhaka. Journal of science foundation. 2012; 10(2): 63-9.
11. Rathod VS , Kasturi, Raut SS. Emergence of multi-drug resistant strains among bacterial isolates in burn wound swabs in a tertiary care centre, Nanded, Maharashtra. India Int J Res Med Sci. 2017;5(3):973-7.
12. Rani vs, Rao Rk, Ravinder s, kanakadurga p. prevalence of extended spectrum beta lactamase (esbl) producing pseudomonas aeruginosa isolates from burns patients. international journal of contemporary medical research 2016; 3 (5):1297-1300.

13. Yasmin T, Yusuf MA, Sayam MAN, Haque R, Mowla G. Status of ESBL producing bacterial isolated from skin wound at a tertiary care hospital in Bangladesh. Scientific research. 2015; 5(4):6.

14. Hasan R, Acharjee M, Noor R. Prevalence of vancomycin resistant Staphylococcus aureus (VRSA) in methicillin resistant S. aureus (MRSA) strains isolated from burn wound infections. Tzu Chi Medical Journal. 2016; 28: 1-5.

15. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, P.A.: Clinical and Laboratory Standards Institute, USA 2017.

16. Tasnim A, Shamsuzzaman AK, Ferdose J, Khan FA. Phenotypic and Genotypic Isolation of MRSA from Burn Wound Infected Patients at a Tertiary Care Hospital in Bangladesh. Bangladesh J Infect Dis 2020;7(1):12-7.

17. Rajbahak S, Shrestha C , Shrestha J, Singh A. Bacteriological changes of burn wounds with time and their antibiogram.- Scientific world. 2014; 12: 312-8.

18. Jobayer M, Rahman M, Akter N, Shareef N, Rana RA, Shamsuzzaman SM. Organisms Isolated from Wound Swab and Pus with their Antibiotic Susceptibility Pattern in a Tertiary Care Hospital of Bangladesh. BMRCB. 2021;47(2):181-7.

19. Joy S, D'souza RC, Shreevidya K , Surlu VR, Suresh S, Jakribettu RP, Baliga MS. Bacteriological Profile of Pathogens in Burns Unit of a Tertiary Care Center: A Retrospective Observational Study.National library of Medicine.2018;32(12):345-9.

20. Islam MS, Ahmed MF, Rahman. Incidence of Methicillin Resistant Staphylococcus aureus in Burn Patients Admitted to Burn Unit, Dhaka Medical College Hospital. Advances in Microbiology. 2013; 3: 498-503.

21. Khurram MF, Maurya SK, Maurya RK, Yaseen M. Bacteriological profile and antibiotic sensitivity patterns of burn wound in delayed presenting cases of burn at a tertiary care centre in India . Int J Community Med Public Health. 2018 ;5(8):3315-20.

22. Fatema K, Sultana S, Ali MH, Akter T, Kobra KT, Islam S. Detection of Pathogenic Microorganisms from Burn Patients Admitted in Tertiary Medical College Hospital and Their Antimicrobial Patterns. Open Journal of Medical Microbiology, 2021; 11: 58-67.

23. Chaudhary NA, Munawar MD, Khan MT, Rehan K, Sadiq A. Epidemiology, Bacteriological Profile, and Antibiotic Sensitivity Pattern of Burn Wounds in the Burn Unit of a Tertiary Care Hospital. PMC2019; 11(6):4794.

24. Bandekar N, Vinodkumar CS, Basavarajappa KG, Prabhakar PJ, Nagaraj P. Beta lactamases mediated resistance amongst gram negative bacilli in Burn infection. Int J Biol Med Res. 2011; 2(3): 766-70.

25. Jobayer M, Rahman M, Akter N, Shareef N, Rana RA, Shamsuzzaman SM. Antimicrobial Susceptibility Pattern of Extended-spectrum Beta-lactamases Producing Organisms Isolated in a Tertiary Care Hospital, Bangladesh. Int J Appl Basic Med Res. 2017; 7(3): 189–192.

26. Keerthi S , Binoy Kurian , Sathiavathy KA. Antibiotic susceptibility patterns of bacterial isolates in burn wound infection in a tertiary care hospital. Journal of International Medicine and Dentistry. 2017; 4(3): 95-104.