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Isolation and Identification of Bacterial Burn Wound Infection and Their Antimicrobial Resistance

Ahlam Ali Majbas[1], Abdullah Rashad Zaghir[2], Amna Faaz Naser[3], Hadeel Abeer Rady[4], Noor alhuda Khalid Naji[5], Rawan Ibrahim Hammad[6], Nour Mohesen Habib[7]

[1,3,4,5,6]University of Fallujah, College of Applied Science, Department of Pathological Analysis, Iraq

[2,7]Dhi-Qar University, College of Science, Department of Pathological Analysis, Iraq

Abstract:

Burns constitute a major role in mortality and morbidity in the whole world, whether accidental, suicidal or homicidal. Burn injuries are among the most devastating of all injuries and a major global public health crisis Burns are the fourth most common type of trauma worldwide, following traffic accidents, falls, and inter personal violence. Bacterial infections are serious problem among burns patients and P.aeruginosa has emerged as the commonest organism causing infection and is resistant to most of the antibiotics.

Keywords: Burn Wound, Infection, Bacteria, Antimicrobial Resistance

Corresponding Author: Ahlam Ali Majbas†, University of Fallujah, College of Applied Science, Department of Pathological Analysis, Iraq

Introduction

Approximately 90 percent of burns occur in low to middle income countries, regions that generally lack the necessary infrastructure to reduce the incidence and severity of burns (Nasih Othman, 2010). Despite of them modernization, the domestic fire is the major cause of the burns with maximum involvement of females and the accidental injury the main cause (Santos et al., 2016). Generally, microorganisms will colonize and grow quickly after burns due to the loss of the skin barrier(Mayhall, 2012). In burn patients, potential biomarkers can be used clinically to identify infections and sepsis, they can also be used to predict the survival of injuries, monitor the severity of injuries organ function or wound healing. There are several risk factors which facilitate microbial colonization and infection, including age and comorbidities, burn wound size, impaired immunity (e.g., hyperglycemia hypermetabolic response), and medical

measures (e.g., use of invasive catheters, transfusion, delays in burn wound excision) etc (Mayhall, 2012) (Mozingo and Pruitt, 2014). The microbial colonizers or pathogens affecting burn patients include bacteria and fungi(Siegel et al,. 2007; Weber and McManus, 2004) The most common Gram-positive bacteria implicated in burn wound infections include Staphylococcus spp., Enterococcus spp., and beta hemolytic Streptococcus group A. Among that group, Staphylococcus aureus continues to be one of the most important bacterial cause of bum wound infections (Ronat et al, 2014; Devrim et al, 2017; Ramirez-Blanco etal, 2017). The most frequently isolated Gram-negative bacteria from patients with burn wounds include Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella spp., Stenotrophomonas spp. Escherichia coli, and Enterobacter cloacae (Mayhall, 2012; Mozingo et al., 2014; Ronat et al., 2014; Merchant et al., 2015). Patients with severe burns are more prone to infections caused by multiple drug-resistant organisms (MDRO); common examples include methicillin- resistant Staphylococcus aureus (MRSA), vancomycin resistant Enterococcus (VRE), MDR Pseudomonas spp. and Acinetobacter spp (Ronat et al., 2014; Siegel et al., 2007). The care and treatment of these patients have been quite challenging. If some of the infection control measures are neglected these pathogens may even cause an outbreak in burn units(Lachiewicz et al., 2015). These patients with burns. Additionally, the cost of medical care for burn patients is substantial (Chen et al., 2009; Hop et al., 2014). In burns patients infections arises from multiple sources. Burn wounds become initially colonized and infected with Gram positive bacteria mainly Staphylococci, these Gram Positive bacteria such as Staphylococci are found during first post burn days that are superseded during the second week by Gram Negative bacteria. Pseudomonas aeruginosa may be the dominant isolate from burn wound colonization and infection Following 2nd week of burns(Manson et al., 2009). The resistance pattern of bacteria against antimicrobial drugs is increasing day by day. The particular level of resistance of bacteria against antibiotics varying from one location to any other and resistance of bacteria against antibiotic becoming global problem (Lari, 2000).

Materials and Methods:

This prospective study was concluded 50 patients admitted in burn unit . A total of 50 surface swabs were taken using standard methods and cultured for growing of the bacteria which were subjected to antibiotic sensitivity testing. Chronic wound for the purpose of this study was defined as any burn wound with skin loss which failed to heal or epithelize naturally within3 weeks from the date of injury. The required data of burn patients including age, sex, season, causes of burns, burn size, manner (way) of burning based on intentional (on purpose) or unintentional (casually). Specimens were cultured on appropriated culture media including MacConkey agar, Blood agar, Nutrient agar and Eeosin Methylen Blue Agar. The cultures were incubated in 37°C for 24-48 h and then the colonies were removed for further study. Biochemical and Culture characterization of the isolates were verified for identification purposes (Koneman et al,1997). In essential cases, specific bacteria was used for precise identification of bacteria type. In order to detection of the susceptibility of isolates to antimicrobial drugs, all isolates were tested by Viteck system (Biomerieux company) 8 antibiotics including Ciprofloxcin, Amikacin, Impenem, Gentamicin, Ticarcillin, Tobramycin, Trimethoprim, Ceftazidin.

Results:

The bacterial species were diagnosed by morphological and biochemical tests conducted on the isolates under study, after cultivation and purification on different culture media and the results were as follows: Staphylococcus isolates were gram-positive and arranged in regular clusters, while gram- negative bacteria appeared in the form of short, gram-negative bacilli Figure(2).

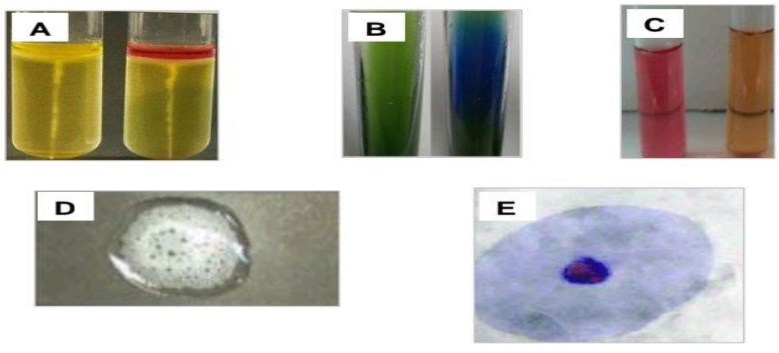
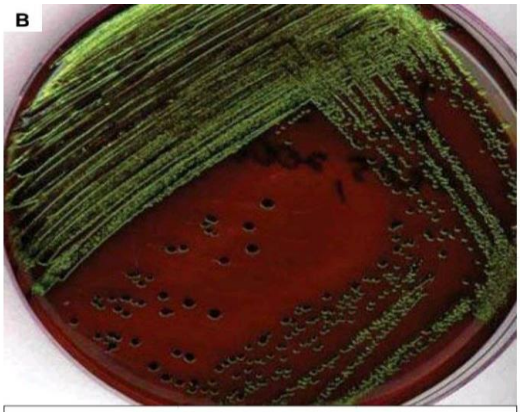


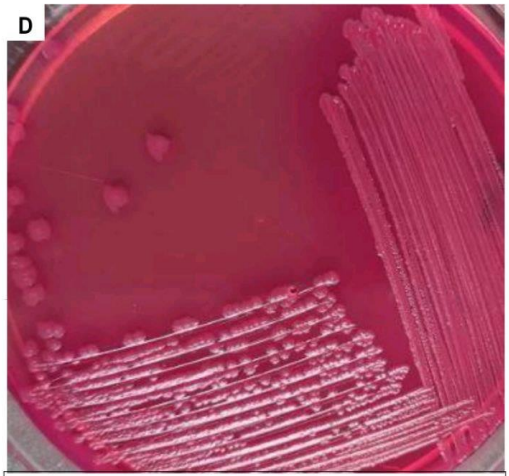
Figure (1): The results of biochemical test A.Indol test(+ve)(-ve). B. Simmon Citrate test, C. Urease test (+ve,(-ve), D.Catalase test (+ve), E.Oxdiaes test

A- P.miribilis colonies growing on blood agar medium



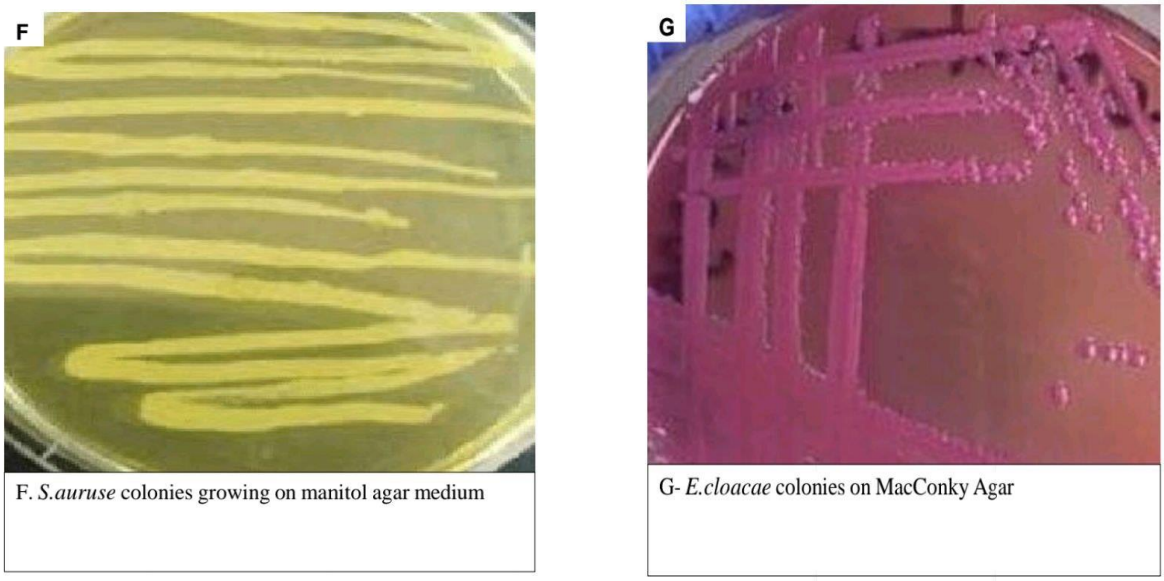
B- *E.coli* colonies growing on (EMB) medium

C-*P.aeruginosae* colonies growing on nutrient agar medium



D- *K. pneumoniae* colonies on MacConky Agar

E- *E.coli* colonies on MacConky Agar



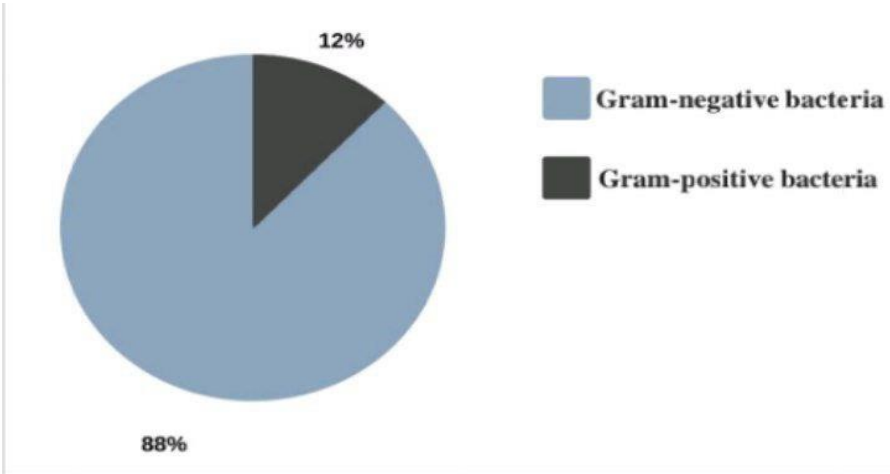
F. *S.auruse* colonies growing on manitol agar medium

G- *E.cloacae* colonies on MacConky Agar

Figure (2): different species of bacteria growing on different growth mediums

During the present study, patients with suspected burn wound infections had their wounds. A total of 50 pathogenic and opportunistic bacteria were identified. In all 50 isolates (88%) were Gram-negative bacilli and 6 (12%) were Gram- positive cocci Figure(3). The predominant bacteria isolated from the infected wounds was Pseudomonas aeruginosa (38%) followed by Klabseilla pneumoniae (20%), Eschreish coli (16%), Enterobacter cloacae (10%), Staphylococcus aureus (8%), Protus miribilla (4%), Staphylococcus epidermis (4%). (Table 1). These bacterial species are considered opportunistic pathogens, and they rarely cause disease in healthy people, but they are highly virulent in patients with weak defense mechanisms, causing bacteremia. therefore, the contamination in hospitals because these pathogens have a pathological effect to deteriorate the state of those sleeping there.

The results of the current study showed that P. aeroginosa isolates were 38%, which is similar to what was found by Boyer et al. (2015), with an isolate rate of 43 (41.3%). Also, this study agrees with what was stated Okon et al. (2014) as an it obtained isolate rate of 30.1%.



Figure(3) Distribution of positive and negative isolates of Gram stain and their percentage

The prevalence of this bacteria may be due to its resistance to antibiotics and disinfectants, and the transformation of the burn area into a suitable medium for the growth of these bacteria due to the weak resistance of the skin tissues subject to burning and its damage. The number and medical supplies, in addition to the severity of overcrowding in the burn unit sometimes.

Table (1) Frequency distribution of isolated microorganisms from burn.

Isolates	Frequency of isolates	Percentage
Pseudomonas aerogenosa	19	38%
Klabseilla pneumonia	10	20%
Eschresch coli	8	16%
Enterobacter cloacae	5	10%
Staphylococcus aureua	4	8%
Proteus mirabilis	2	4%
Staphylococcus epidermdis	2	4%

This study did not agree with the study of Kandati et al., (2015) and others. They found that there was an increase in the percentage of isolates of S.aureus bacteria in the first place, with an isolation rate of 39.8%, then followed by P.aeroginosa bacteria with an isolation rate of 35.3%. The reason may be due to the number of samples taken. Included in the study or according to the geographical location, it varies from one location to another and from one hospital to another.

In a study conducted by Adugna et al. (2015), in which it was confirmed that the rate of isolation of E.coli bacteria was 15%, which is close to the ratio in the current study, 11.1 E.coli bacteria constitute a high percentageamong the Gram-negative bacteria naturally present in the body, and infection may be As a result of infection or acquired infection from hospitals, due to its high percentage in most hospitals (Bowler and Davies, 1999)

Out of 50 infected patients, 36% were males and 64% were females. (Figure 4) It is identical to study (Hakim et al., 2016) with a rate of isolation of 34% for males and 66% for females. This is due to the nature of female work in homes and their preoccupation with housework, especially with regard to cooking or directly near sources of fire and hot liquids and vapors. Females are at risk twice more than men because of kitchen accidents and domestic violence. The results of the current study also agreed with what was found Panjeshahin et al (2011), that burns afflict women more than females, due to direct contact with sources of fire, such as kerosene-powered cookers, which often lead to flame burns. As it is shown in Table 2 the causes burns in patients, boiled water (44%), Fire by gas flames (22%), Fire by gasoline flames(18%), Hot liquids (10%) and Electrical shock (6%), the results of this study came close to what was found by Bujok. (2010). who stated that the highest burning rate was with boiling water by 23.4%, then burning by gas flames by 14.5%, and the lowest burning rate was for electrical burns by 4%. The results of this study also agreed with what was found by Ozkurt et al., (2012). The highest insulation rate was for boiled water with an isolation rate of 65.5%, then burns of gas flames with an isolation rate of 13.6%, most burn injuries occur at home or at work, and in the United States of America, the most common causes of burns are fire or flames by 44%, hot objects 9%, electricity 4%.

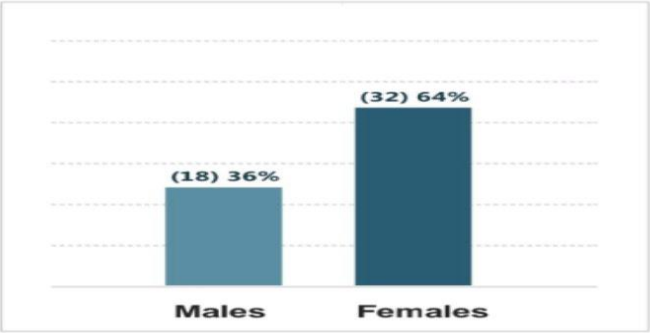


Figure (4) The distribution of the injured according to the gender

Table (2) The frequency distribution of the causes of burns in patients.

Causes of burn	Frequency	Percentage
Boiled water	22	44%
Fire by gas flim	11	22%
Fire by gasoline flim	9	18%
Hot liquids	5	10%
Electrical shock	3	6%

Frequency of the manners of burning based on intention or accidentally was as follow: (86%) (43cases) of patients were burned accidentally and (14%) (7 cases) were intention. (Figure 5) The results of the current study agreed with what was found by Peck, (2012), and this study was also identical to what was mentioned (Mashouf and Hashemi, (2006), and the percentage of unintended injuries was 73.6%, while the percentage of intended injuries was 26.4%.

Intended burns are among the common causes of burns. Or it can be called intentional burns that occur in a significant proportion of young women and that occur due to social and economic problems

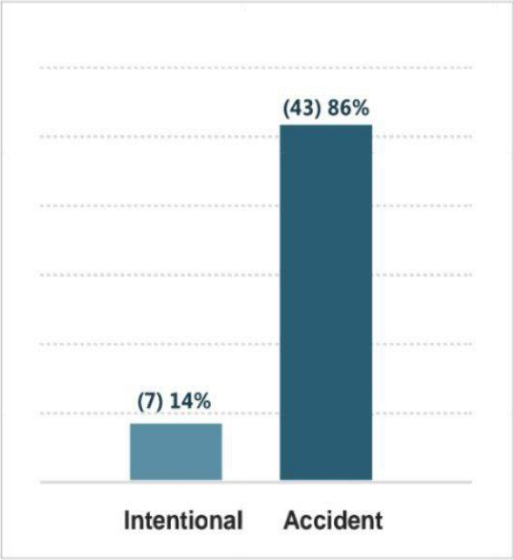
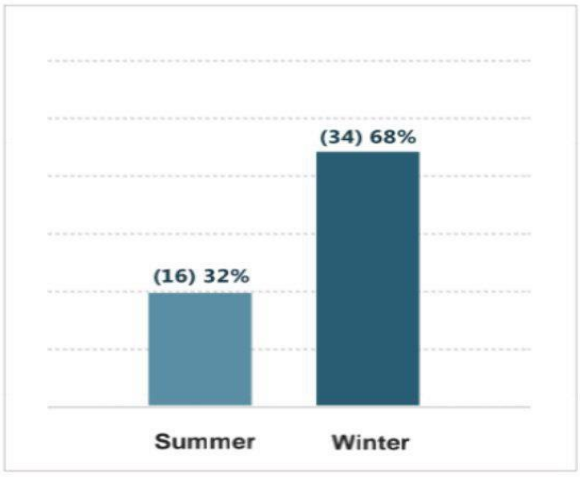


Figure (5) The distribution of the injured accident and intentional

proportion of cases were among individuals who live in country increased by 37 injured 74% while the injuries the city has reached 13 cases of burn injury by 26% (Figure 7) This study was identical to what was found by Attia et al.

(1997), and this study was similar to what was found by Haralkar et al., (2011). The reason for this may be due to the standard of living, low socio- economic status, and the use of traditional cooking utensils. in preparing food.



Figure(6) The distribution of the injured according to the seasons

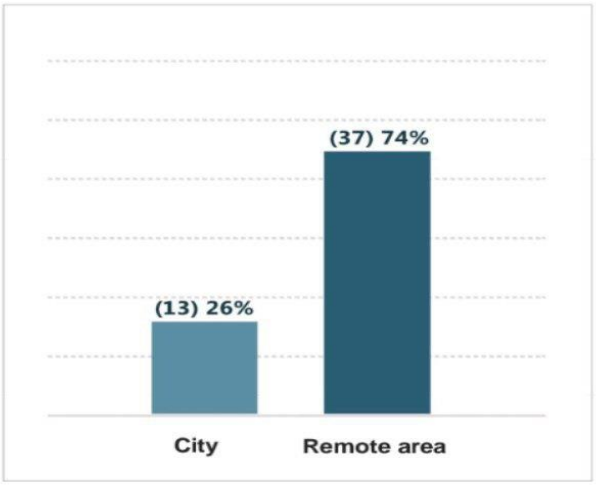


Figure (7) The distribution of the injured according to the location.

An antibiotic sensitivity test was conducted using the Vitek 2 device where the sensitivity of bacterial isolates to eight types of common antibiotics was tested, and these antibiotics are: Amikacin, Ceftazidin, Ciprofloxacin, Gentamicin, Imipenen, Ampicilin, cefepime, and Trimethoprm. The results of the current study showed, that the most effective antibiotics against both gram-negative and gram-positive bacteria are Amikacin, Ciprofloxacin, Ceftazidin Table(4,5). The resistance ratio to Ciprofloxacin, varied from one bacteria to another, as the current results showed that P. aeruginosa was resistant to this antibiotic by 84%, is due to the fact that P. aeruginosa is one of the most important causes of hospital-acquired infections, especially infections that are associated with the length of treatment and the survival of the patient suffering from complications of the disease. The results of the current study agreed with (François et al., 2016), agreed this study with a study Performed by Abdullah et al. (2010) who found that there is a decrease in the level of anti-Ciprofloxacin.

The results of the current study showed that the isolates that were resistant to Cirprofloxacin and to the rest of the other types of antibiotics used in the study because this antibiotic is one of the modern antibiotics specially

manufactured to treat infections caused by P. aeruginosa bacteria. which was resistant to ciprofloxacin, and to the rest of the other types of quinolones used in the study.

The results of the current study showed that K.pneumoniae were sensitive to Ciprofloxacin by 32%. and it showed its resistance to the antibiotics Ceftazidin, Trimethoprim-sullphamethoxazole, and Gentamicin, as the results of the current study agreed with what was found by Lob et al. (2016), which indicated the resistance of bacteria to the above-mentioned antibiotics, while these results did not agree with what the researchers found (Finlayson and Brown, 2011.(

) Iacchini et al., 2019) showed in a study on E.coli bacteria to increasing resistance to antibiotics, where the percentage of resistance to the antibiotic Cirprofloxacin reached 84%, which was identical with the current study. The resistance is 86%, and then the antibiotic Gentamicin comes with a resistance rate of 84%. The results also agreed with what was stated (Varela et al., 2021; Bonkat et al., 2013) where the bacteria were resistant by 89%, 100%, and agreed with what was stated in a study in Spain conducted by researchers (Hrbacek et al., 2020). E.cloacae bacteria, it showed resistance to Topramycin, Tecarcillin, and Gentamicin, and the results of this study were similar to a study conducted by Thiolas et al., (2005). The results of the current study showed that P.mirabilis was resistant to the antibiotics, Ceftazidin Tecarcilin, Topranycin It was sensitive to the anti- ciprofloxacin, and the results of this study were identical to the results of Nccls. (2003). As for S.aureus bacteria, the results of the current study showed that it is close to the results of Kolar et al., (2009), in which it was mentioned that it is resistant to both Tecarcilin, Gentamycin by 50% and 50%, respectively.

Table (4) Sensitivity and resistance of gram-negative bacteria to some antibiotics.

P.miribilis			*E.cloaca*			*E.coli*			*K.pneumoniae*			*P.aeruginosa*			Antibiotics
I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	
0	25%	75%	0	16%	84%	0	38%	62%	0	25%	75%	0	0	100%	Amikacin
0	50%	50%	0	100%	0	0	16%	84%	0	32%	86%	0	16	84%	Ciprofloxacin
0	0	100%	50%	0	50%	0	50%	50%	0	0	100%	0	0	100%	Ceftazidin
0	0	100%	0	44%	66%	0	15%	85%	0	0	100%	0	0	100%	Gentamicin
0	25%	75%	0	17%	83%	0	43%	67%	0	0	100%	0	0	100%	Imipenem
0	0	100%	0	0	100%	0	0	100%	0	0	100%	0	0	100%	Cefepime
0	25%	75%	25%	0	75%	0	0	100%	0	0	100%	0	0	100%	Ampicillin
0	0	100%	0	50%	50%	0	50%	50%	0	0	100%	0	0	100%	Trimethoprim

Table (5) Sensitivity and resistance of *S.aureus* bacteria to some antibiotics.

S.epidermedis			*S.aureus*			Antibiotics
I	S	R	I	S	R	
	0	100%	0	0	100%	Ciprofloxacin
0	0		0	8%	92%	Erythromycin
0	50%	50%	0	50%	50%	Gentamicin
0	0	100%	50%	0	50%	Levofloxacin
0	25%	75%	0	16%	84%	Tobramycin
0	0	100%	0	68%	32%	Trimethoprm
0	100%	0	0	100%	0	Vancomycin

Conclusion:

Bacterial infections are serious problem among burns patients and P.aeruginosa has emerged as the commonest organism causing infection and is resistant to most of the antibiotics.

References:

1. Othman, N. (2010). Epidemiology of burn injuries in Sulaymaniyah province of Iraq (Doctoral dissertation, University of Nottingham)pp.56.
2. Santos, J. V., Oliveira, A., Costa-Pereira, A., Amarante, J., & Freitas, A. (2016). Burden of burns in Portugal, 2000-2013: a clinical and economic analysis of 26,447 hospitalisations. Burns, 42(4), 891-900.
3. Mayhall, C. G. (2012). Hospital epidemiology and infection control. Lippincott Williams & Wilkins..
4. Chen, Y. Y., Wu, P. F., Chen, C. S., Chen, I. H., Huang, W. T., & Wang, F. D. (2020). Trends in microbial profile of burn patients following an event of dust explosion at a tertiary medical center. BMC infectious diseases, 20(1), 1-11.
5. Siegel JD, Rhinehart E, Jackson M, Chiarello L,. (0226). Healthcare Infection Control Practices Advisory C. Management of multidrug-resistant organisms in health care settings.. Am J Infect Control. 2007;35:S165-93.
6. Weber, J., McManus, A., & Nursing Committee of the International Society for Burn Injuries. (2004). Infection control in burn patients. Burns, 30(8), A16-A24.
7. Ronat, J. B., Kakol, J., Khoury, M. N., Berthelot, M., Yun, O., Brown, V., & Murphy, R. A. (2014). Highly drug-resistant pathogens implicated in burn-associated bacteremia in an Iraqi burn care unit. PloS one, 9(8), e101017 .
8. Devrim, İ., Kara, A., Düzgöl, M., Karkıner, A., Bayram, N., Temir, G., & Hoşgör, M. (2017). Burn-associated bloodstream infections in pediatric burn patients: time distribution of etiologic agents. Burns, 43(1), 144-148 .
9. Ramirez-Blanco, C. E., Ramirez-Rivero, C. E., Diaz-Martinez, L. A., & Sosa-Avila, L. M. (2017). Infection in burn patients in a referral center in Colombia. Burns, 43(3), 642-653.
10. Merchant, N., Smith, K., & Jeschke, M. G. (2015). An ounce of prevention saves tons of lives: infection in burns. Surgical Infections, 16(4), 380-387.
11. Siegel, J. D., Rhinehart, E., Jackson, M., & Chiarello, L. (2007). 2007 guideline for isolation precautions: preventing transmission of infectious agents in health care settings. American journal of infection control, 35(10), S65-S164.
12. Lachiewicz, A. M., van Duin, D., DiBiase, L. M., Jones, S. W., Carson, S., Rutala, W. A., & Weber, D. J. (2015). Rates of hospital- associated respiratory infections and associated pathogens in a regional burn center, 2008-2012. infection control & hospital epidemiology, 36(5), 601- 603..
13. Chen, Y. Y., Chou, Y. C., & Chou, P. (2005). Impact of nosocomial infection on cost of illness and length of stay in intensive care units. Infection Control & Hospital Epidemiology, 26(3), 281-287 – ..
14. Chen, Y. Y., Wang, F. D., Liu, C. Y., & Chou, P. (2009). Incidence rate and variable cost of nosocomial infections in different types of intensive care units. Infection Control & Hospital Epidemiology, 30(1), 39-46..
15. Hop, M. J., Polinder, S., van der Vlies, C. H., Middelkoop, E., & van Baar, M. E. (2014). Costs of burn care: a systematic review. Wound repair and regeneration, 22(4), 436-450.
16. Manson, W. L., Pernot, P. C. J., Fidler, V., Sauer, E. W., & Klasen, H. J. (1992). Colonization of burns and the duration of hospital stay of severely burned patients. Journal of hospital Infection, 22(1), 55-63.
17. Lari, A. R., & Alaghehbandan, R. (2000). Nosocomial infections in an Iranian burn care center. Burns, 26(8), 737-740.

18. Boyer, F., Couturier, É., Pouliquen, O., & Guazzelli, É. (2015.(Suspensions in a tilted trough: second normal stress difference. Journal of Fluid Mechanics, 686, 26-39.

19. Okon E., Uboh, F. I. E., & Ekong, M. B. (2014). Effect of aqueous extract of Psidium guajava leaves on liver enzymes, histological integrity and hematological indices in rats. Gastroenterology Research, 3(1), 32.

20. Kandati, S., Flack, K. L., Agarwal, P., & Selfe, T. K. (2015). The association of restless legs syndrome to history of gestational diabetes in an Appalachian primary care population. Journal of Clinical Sleep Medicine, 11(10), 1121-1130.

21. Adugna, A., Kibret, M., Abera, B., Nibret, E., & Adal, M. (2015.(Antibiogram of E. coli serotypes isolated from children aged under five with acute diarrhea in Bahir Dar town. African health sciences, 15(2), 656-664.

22. Bowler, P. G., & Davies, B. J. (1999). The microbiology of infected and noninfected leg ulcers.

23. Hakim, E. H., Achmad, S. A., Juliawaty, L. D., Makmur, L., Syah, Y. M., Aimi, N., & Ghisalberti, E. L. (2016). Prenylated flavonoids and ... related compounds of the Indonesian Artocarpus (Moraceae). Journal of Natural Medicines, 60(3), 161-184.

24. Panjeshahin, M. R., Lari, A. R., Talei, A. R., Shamsnia, J., & Alaghehbandan, R. (2011). Epidemiology and mortality of burns in the South West of Iran. Burns, 27(3), 219-226.

25. Bujok, G. (2010). Haemodynamic Disorders in the Course of Electrical Burns. A Preliminary Report. Annals of Burns and Fire Disasters, 19(1), 36.

26. Prestinaci, F., Pezzotti, P., and Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. Pathogens and global health, 109(7), 309.

27. Talaiekhozani, A., Alaee, S., and Ponraj, M. (2015). Guidelines for quick application of biochemical tests to identify unknown bacteria. AOBR, 2(2), 65-82.

28. Lob, S. H., Nicolle, L. E., Hoban, D. J., Kazmierczak, K. M., Badal, R. E., and Sahm, D. F. (2016). Susceptibility patterns and ESBL rates of Escherichia coli from urinary tract infections in Canada and the United States, SMART 2010-2014. Diagnostic microbiology and infectious disease, 85(4), 459-465.

29. Hrbacek, J., Cermak, P., and Zachoval, R. (2020). Current antibiotic resistance trends of uropathogens in Central Europe: Survey from a Tertiary hospital urology department 2011-2019. Antibiotics, 9(9), 630.#

30. Varela, M. F., Stephen, J., Lekshmi, M., Ojha, M., Wenzel, N., Sanford, L. M., and Kumar, S. H. (2021). Bacterial Resistance to Antimicrobial Agents. Antibiotics, 10(5), 593.#

31. Iacchini, S., Korbekandi, H., Mirmohammadi, S. V., and Zolfaghari, B. (2019). Synthesis of silver nanoparticles: chemical, physical and biological methods. Research in pharmaceutical sciences, 9(6), 385.

32. Francois, M., Hanslik, T., Dervaux, B., Le Strat, Y., Souty, C., Vaux, S., and Rossignol, L. (2016). The economic burden of urinary tract infections in women visiting general practices in France: a cross-sectional survey. BMC health services research, 16(1), 1-