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Prevalence of gram negative bacteria in infected burn wounds and antibiotic susceptibility pattern; A study of stable burn patients.

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ABSTRACT… Objective: To study prevalence of various gram negative bacteria in infected burn wounds among stable burn patients reporting to out-patient department on follow-ups. **Study Design:** Cross Sectional study. **Setting:** Department of Pathology, Sahara Medical College Narowal. **Period:** January to June 2020. **Material & Methods:** Patients with burn wounds with clinical signs and symptoms of infection but vitally stable, wound less than one month old involving < 20% body surface, reporting to out-patient door of study institution on follow-ups were enrolled into the study. Swabs of infected wounds were taken and sent for bacterial culture and sensitivity to the pathology department of the institution, where micro flora were isolated and their antibiotic susceptibility pattern was determined using standard techniques. Consent was taken from patients before including them in study. **Results:** Total 210 cases were studied. Gram negative bacteria were isolated from 190 cases, out of them 30% were oxidase positive ad 70% were oxidase negative. Most common organism isolated was Pseudomonas Aeruginosa (30%), followed by Proteus Sps. (25.3%) and Entero bacter Sps. (15.8%) etc. **Conclusion:** Among gram negative bacteria pseudomonas is a major isolated organism from infected burn wounds having high susceptibility to imipenem and cefepime.

Key words: Burn Wounds, Culture and Sensitivity, Gram Negative Bacteria, Infection.

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INTRODUCTION

Burn injuries are severe form of trauma requiring specialized care to reduce morbidity and mortality. According to American Burn Association report nearly 0.5 million patients annually seek treatment for burn injuries and about 60,000 patients are admitted in hospitals due to these injuries annually, causing 60%-75% burn related deaths.[1] Burn injuries cause immunosuppression so predisposing to opportunistic infections in burn patients.[2] Burn wounds infection is very common and it require proper management and good antibiotic coverage. Moist burn wound containing dead, devitalized tissue is ideal for the growth of bacteria, hence chances of wound infection and sepsis persist until complete wound healing.[3] Most of the first and second degree burn wounds do not progress to serious infection but serious infections are common in third degree burns and burns involving large total body surface area (TBSA). Burn injuries destroy physical skin barrier, metabolic, sensory and immunological functions of skin as well.[4] These wounds serve as portal of entry for pathogens into the body. Burn eschar provides shelter for the growth of microbes and inhibiting infkux of antimicrobial agents and immune cells. Burn wound infections may occur from gram positive and gram negative bacteria both.[5] Hospital acquired wound infections are also common due to various invasive procedures. Staphylococcus aureus is a major cause of burn wounds infection followed by pseudomonas.[6] Pseudomonas aeruginosa causes severe wound infection among burn patients via cross contamination of wounds, having high resistance and pathogenicity and associated with high morbidity and mortality rate. It causes very serious infections difficult to treat.[7] Gram negative enteric bacteria produce pus in burn wounds and destroy surviving underlying skin cells so converting

partial thickness burns into full thickness, also they don't let skin grafts survive.[8] They produce toxins which when absorbed into blood may cause sepsis leading to death.[9] A major issue in treating such immunocompromised patients with severe burn wounds is increasing antibiotic resistance of pathogens causing wound infections.[10] Hence common infections are becoming difficult to treat with long recovery period with the passage of time. Now it is need of the time to determine bacterial antibiotic sensitivity before starting antibiotic treatment. Swabs should be taken from wounds and send for culture and antibiotic sensitivity pattern.

MATERIAL & METHODS

It is cross sectional study conducted at pathology department of Sahara Medical College Narowal. Sample size was calculated using WHO sample size formula. Non-randomized convenient sampling technique was used for sample selection. Patients with first, second or third degree burn wounds less than 30 days passed, involving body surface area <20%, vitally stable and having clinical signs and symptoms of wound infection like pus, discharge, bleeding and pain etc. were enrolled into the study. Children, patients with co-morbidities, wounds older than one month, already taking any antibiotic treatment in last two weeks, were not included in the study.

A proforma was designed to document all relevant data of each patient like biodata name, age, gender, address, mode of bur injury, duration, any previous treatment taken, positive findings on wound examination etc. Swabs from infected part of burn wounds were taken and preserved in Stuart's transport medium and sent to the hospital laboratory. Bacterial cultures were incubated at 37 Degree Celsius on Macconkey's agar, blood agar and nutrient agar for whole night.[11] After incubation, isolates were identified by their colonial morphology and gram staining. Gam negative bacterial were further subjected to oxidase test and further biochemical tests were performed for confirmation. According to guidelines of National Committee for Clinical Laboratory, antibiotic susceptibility patter of isolates was determined using Kirby Bauer Method.[12] Data collected was analyzed using SPSS-20. Chi square test was applied, taking confidence interval 95% and margin of error 5%. Means and standard deviation were determined for quantitative variables and frequency, percentage for qualitative variables. P-value <0.05 was considered statistically significant.

RESULTS

Total 210 bacterial isolates taken from 70 patients, were sent for culture and sensitivity. Out of them 190 isolates showed gram negative bacteria. They were further subjected to oxidase test, 57(30%) were oxidase positive and 133(70%) were oxidase negative. Age of patients was 15-60 years with mean age of 32.74± 9.36 years. Out of gram negative bacteria pseudomonas aeruginosa was most prevalent bacteria found in 57(30%) isolates, followed by proteus (25.3%) and Enterobacter (15.8%).

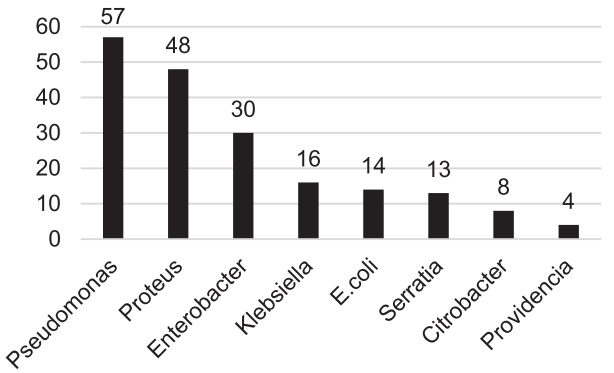

Figure-1. Frequency of Gram-negative bacteria in bacterial isolates in study group. (n=190)

Imipenem was more active against gram-negative bacteria than other drugs with overall activity of 94%, followed by cefepime (70.2%), piperacillin (51.8%), cefuroxime (70.2%), ceftriaxone (37.4%), aztreonam 33.5%, augmentin (22.3%) and amoxicillin with overall activity of 14.8%. Pseudomonas sensitivity to imipenem was seen sufficiently while resistance was shown to other antibiotics. Hence imipenem is much effective in treating gram negative bacterial infections.

Drug Name	Pseudo.	Prot.	Enter.	Kleb.	E.coli	Serra.	Citro.	Prov.	Overall Activity of a Drug	P-Value
IMP	44 (77.2%)	46 (95.8%)	30 (100%)	15 (93.7%)	11 (78.6%)	10 (77%)	07 (87.5%)	04 (100%)	94%	0.453
CEF	27 (47.4%)	29 (60.4%)	21 (80%)	07 (43.7%)	12 (85.7%)	09 (69.2%)	06 (75%)	04 (100%)	70.2%	0.446
PIP	12 (21.1%)	27 (56.2%)	06 (20%)	09 (56.2%)	08 (57.1%)	07 (53.8%)	04 (50%)	04 (100%)	51.8%	0.326
CFU	05 (8.8%)	22 (45.8%)	10 (33.3%)	03 (18.7%)	06 (42.8%)	07 (53.8%)	06 (75%)	04 (100%)	47.3%	0.542
CFT	10 (17.5%)	18 (37.5%)	08 (26.7%)	05 (31.2%)	06 (42.8%)	04 (30.7%)	03 (37.5%)	03 (75%)	37.4%	0.404
CFR	03 (5.3%)	09 (18.7%)	07 (23.3%)	02 (12.5%	05 (35.7%)	04 (30.7%)	03 (37.5%)	04 (100%)	33%	0.370
AZT	6 (10.5%)	16 (33.3%)	10 (33.3%)	02 (12.5%)	05 (35.7%)	04 (30.7%)	03 (37.5%)	03 (75%)	33.5%	0.451
AUG	02 (3.5%)	06 (12.5%)	05 (16.7%)	03 (18.7%)	04 (28.6%)	03 (23.1%)	02 (25%)	02 (50%)	22.3%	0.662
AMX	00 (00%)	05 (10.4%)	03 (10%)	01 (6.2%)	02 (14.3%)	02 (15.4%)	01 (12.5%)	02 (50%)	14.8%	0.253
Total	57	48	30	16	14	13	08	04	190	

Table-I. Antibiotic susceptibility pattern of isolated Gram-negative organisms in study samples. (n=190)

Pseudo. Pseudomonas aeruginosa, Prot. Proteus, Enter. Enterobacter, Kleb. Klebsiella, Serra. Serratia, Citro. Citrobacter, Prov. Providencia, IMP. Imipenem, CEF. Cefepime, PIP. Piperacillin, CFU. Cefotaxime, CFR. Cefuroxime, AZT. Aztreonam, AUG. Augmentin, AMX. Amoxicillin,

DISCUSSION

Pseudomonas aeruginosa is a main pathogen causing burn wounds infection. It has a prolonged survival in hospital settings even for months and producing hospital acquired multi drug resistant infections among admitted patients.[13] In our study pseudomonas was detected in 30% isolates. Our finding is similar to results of other studies showing 25%-30% prevalence of pseudomonas.[14,15] Burn patients with immunocompromised conditions who have received multiple antibiotic regimens previously are prone to multidrug resistant strains of bacteria. With the passage of time antibiotic resistance is developing from low to intermediate and high resistance. Now there is no antibiotic against which resistance has not been developed. This is a major health issue in developing and developed countries.[16]

Main causes of this developing antibiotic resistance is over usage and use of irrelevant antibiotic due to high cost of suitable preferred antibiotic.[17] Beta lactam antibiotics require prolong treatment courses against gram negative bacteria therefore costly and related to poor compliance by the patient. Carbapenems are effective against many strains of multidrug resistant P. aeruginosa, but resistance against them is increasingly reporting now.[18] Mostly, but not all, MDR Pseudomonas aeruginosa respond to imipenem therapy more than other antibiotics, reporting 91% sensitivity.[19] This is comparable to our finding, 77.2% sensitivity of P. aeruginosa against imjpenem. Aztreonam is a beta lactam antibiotic showed poor activity (10.5%) against p. aeruginosa in our study that is similar to the results of a study done by Hogan et al in USA, reporting 28% sensitivity of pseudomonas for aztreonam.[20] Piperacillin show great activity against P. aeruginosa but in our study its role was poor and showed just 21.1% activity. A study conducted in USA reported combined effect of piperacillin and tazobactam as 77.5%[21], while some other studies reported high resistance of

pseudomonas against piperacillin-tazobactam.[22] This difference in sensitivity patter of microbes against antibiotics may be due to different strains of microbes depending on environment and geographical changes, or due to difference in healthcare facilities in different countries. Third generation cephalosporins have been used as broad spectrum antibiotic against many microbial infections for many decades. In recent years, high resistance has been noticed in microbes against these antimicrobial drugs. That may be due to production of enzyme of betalactamases in microbial strains and passed on to next microbial generations. That is the reason of search for new antimicrobial agents. Fourth generation cephalosporins have been found to have great activity against resistant gram negative bacteria, and hence are suitable for treating hospital acquired infections, infected burn wounds and infections among immunocompromised patients.[23] In our study Overall activity of antibiotics against gram negative microbes was found highest by imipenem (94%), followed by cefepime (70.2%) and piperacillin (51.8%).

CONCLUSION

Pseudomonas among gram negative bacteria is a major cause of burn wounds infection, and imipenem is only drug with great activity against them. Burn wounds infection by multiple bacteria with multidrug resistance strains, require a proper drug policy should be implemented in burn units. As isolated bacteria showed multi drug resistance, hence any antibiotic should be started after determining culture and sensitivity pattern.

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2	Khushbu Farva	Data analysis, Data collection, Data analysis.	
3	Ghulam Asghar Bhutta	Data analysis, Data collection, Found additional literature for information, Data composing.	