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BACTERIAL ISOLATION AND THEIR ANTIBIOTIC SENSITIVITY PATTERN FROM PUS AND WOUND INFECTION IN A TERTIARY CARE HOSPITAL

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Abstract

Introduction: Pyogenic infections are characterized by local and systemic inflammation usually with pus formation. (1) A break in the skin can provide entry to the surface bacteria which there to start multiplying locally. The body's defense system produces immune cells in the area to fight against bacteria. In due course, the accumulation of these cells produces pus which is a thick whitish liquid. (2)

Aims and objectives:

1) To determine the microbiological profile of aerobic organisms in pus and wound infection. 2)To determine the antibiotic sensitivity and resistant pattern of the microorganisms.

Method: Culture has been grown from different specimen and antibiotic susceptibility testing done by Kirby bauer disk diffusion method as standard protocol in Microbiology Laboratory. Antibiotic resistance leads to prolonged hospital stays and increased costs in terms of treatment and causes life-threatening infections. The majority of MRSA strains worldwide have become resistant to multiple antibiotics including beta-lactams; tetracyclines; macrolides and more recently fluoroquinolones.(3) Reducing inappropriate antibiotic use is the best way to control resistance.(4). The microbiology laboratory plays a key role in the decision to choose a particular antimicrobial agent. Definitive treatment depends upon the appropriate selection of antibiotics according to the causative agent likely to work on the patient can be done. (5)

Result : Out of 118 pus samples obtained in the microbiology laboratory from various departments of simmer medical hospital for aerobic culture and sensitivity, 77 (66%)samples yielded a positive culture whereas 40 (34%) samples yielded no growth .78 culture positive cases in 28 MDR cases organism isolates were found.

Conclusion: All the isolates were subjected to antimicrobial susceptibility tests. Gram-positive isolates were most susceptible to vancomycin, levofloxacin and teicoplanin. Most gram-negative isolates were sensitive to imipenem, gentamycin, amikacin, levofloxacin, cefoperazone and cefepime.

KEY WORDS:PUS, ANTIBIOTIC SUSCEPTIBILITY, TERTIARY CARE HOSPITAL

Introduction

Pyogenic infections are characterized by local and systemic inflammation usually with pus formation [(1)]. These may be endogenous or exogenous. A break in the skin can provide entry to the surface bacteria which can start multiplying locally. The body's defense mechanism includes bringing immune cells into the area to fight against bacteria. After that, these cells produce thick whitish liquid pus [(2)].

The inappropriate use of antibiotics leads to increase drug resistance pathogens, which lead to a great challenge to health services. Additionally, highly virulent strains quickly adapt to changing environment lead to worsen the situation and draws a matter of concern [(3)].

Different studies have been conducted across the globe from time to time to assess the bacterial profile and antibiotic susceptibility antibiogram in pus samples. Specifically related to the treating physician who needs to start empirical treatment of patients until the lab culture reports are awaited [(4)].

Though the bacterial profile from pus samples is similar most of the time, but antibiotic sensitivity pattern of these isolates increases the chances of resistant pattern bacteria and hence a need for a study of such changing trends. Therefore, a study was conducted in our tertiary care centre to study the changing pattern in antimicrobial resistance in pus isolate. [(5)].

Antimicrobial resistance leads to prolonged hospital stays and increased costs in terms of treatment. Also, the majority of MRSA strains worldwide have become more resistant to multiple antibiotics including beta-lactams; tetracyclines, macrolides and more recently fluoroquinolones [(6)].

Antimicrobial agents are among the most commonly used and misused of all drugs. The unnecessary and unwanted use of widespread use of antimicrobial agents has been the emergence of antibiotic resistance pathogens, which create demand to need for higher antibiotics. The pace of antimicrobial drug development has slowed after research, with only a handful and necessary of new agents have been introduced every year. De-escalation and antibiotic stewardship may best ways to control resistance [(7)].

The microbiology laboratory plays pivotal role in the decision to choose an appropriate antimicrobial agent. Once causative microorganisms are identified in a microbiology laboratories than antibiotics are wisely chosen for patient care [(8)].

AIMS AND OBJECTIVES

To determine the microbiological profile of aerobic organisms in pus and wound infection.

To determine the antibiotic sensitivity and resistant pattern of the microorganisms.

METHODOLOGY:

SPECIMEN: Pus and wound material was collected in a screw-capped container, a firmly stoppered syringe or a sterile swab from patients suspected of wound infection by the clinician and sent to the microbiology lab for culture and sensitivity.

Pus samples from patients collected by clinicians as per standard guidelines from patients of OPD and IPD wards were sent to the Microbiology laboratory for the bacterial isolation and identification along with their antibiotic sensitivity pattern.

Processing of sample:

Smear was prepared on a clean glass slide and heat fixed. Gram stain was done. Smears were then examined under oil immersion objective for various morphological types of organisms. Then inoculated on blood gar and Mac Conkey agar plates were incubated at 37°c for 24 hours to 48 hours in aerobic conditions.

After incubation on isolation of gram-positive cocci, various tests like catalase and coagulase tests were performed. Gram negative bacilli were identified using biochemical tests IMViC, Oxidase and TSI agar slant.

The antibiotic sensitivity pattern of the microorganism to various antibiotics was determined using Kirby- Bauer method on Muller Hinton agar and the results were interpreted as per clinical laboratory standard (CLSI) guidelines.

In this method McFarland standard of the test bacterium is prepared and inoculated on the surface of a solid medium Muller Hinton agar or Nutrient agar as a lawn plate method by spreading with a cotton swab.

After drying the plate (37°C for 30 minutes) antibiotic discs (4-6 per 9 cm plate) are applied with sterile forceps.

After overnight incubation, the sensitivity pattern is determined by measuring the zones of inhibition of growth around the disc. Growth will be inhibited around the disc containing antibiotics to which the bacterium is susceptible but not around those to which it is resistant.

The zone diameter is affected by many factors, such as the diffusibility of the drug, disc concentration, nature and composition of the medium, its thickness and presence of inhibitory or stimulatory substances, pH and time of incubation.

There are several recommendations regarding the antibiotic concentration to be used a disc. The disc concentration recommended by CLSI guidelines that are internationally acceptable. The other guidelines used are EUCAST. The interpretive guidelines for the disc diffusion test permit the user to make an approximation of the MIC for each of the antimicrobial agents, with zone diameters as determined by the disc diffusion technique[(13)]

The following antibiotics (Hi-Media disc in mcg) were tested

Table 1: Drugs used for gram positive organisms

Sr.no.	**Antibiotic**	**Symbol**	**Concentration**
1	Penicillin	P	10 mcg
2	Cefoxitin	CX	30 mcg
3	Cefazolin	CZ	30 mcg
4	Cefuroxime	CXM	30 mcg
5	Teicoplanin	TEI	30 mcg
6	Linezolid	LZ	30 mcg
7	Vancomycin	VA	30 mcg
8	Amoxyclav	AMC	30 mcg
9	Ciprofloxacin	CIP	5 mcg
10	Levofloxacin	LE	5 mcg
11	Erythromycin	E	15 mcg
12	Co-trimoxazole	COT	25 mcg

Table 2: Drugs used for Gram Negative Organisms

Sr.no.	**Antibiotic**	**Symbol**	**Concentration**
1	Ampicillin	AMP	10 mcg
2	Co-trimoxazole	COT	25 mcg
3	Gentamicin	GEN	10 mcg
4	Amikacin	AK	30 mcg
5	Ciprofloxacin	CIP	5 mcg
6	Levofloxacin	LE	5 mcg
7	Cefuroxime	CXM	30 mcg

8	Cefoperazone	CPZ	75 mcg
9	Cefepime	CPM	30 mcg
10	Imipenem	IPM	10 mcg
11	Piperacillin/tazo bactam	PIT	100/10 mcg
12	Amoxyclav	AMC	10 mcg

RESULT:

Out of 118 pus samples obtained in the microbiology lab from various departments of smimer medical hospital for aerobic culture and sensitivity, 78 (66%) samples yielded a positive culture whereas 40 (34%) samples yielded no growth showing on Table 3.

Figure 1: Micro-organisms growth wise distribution

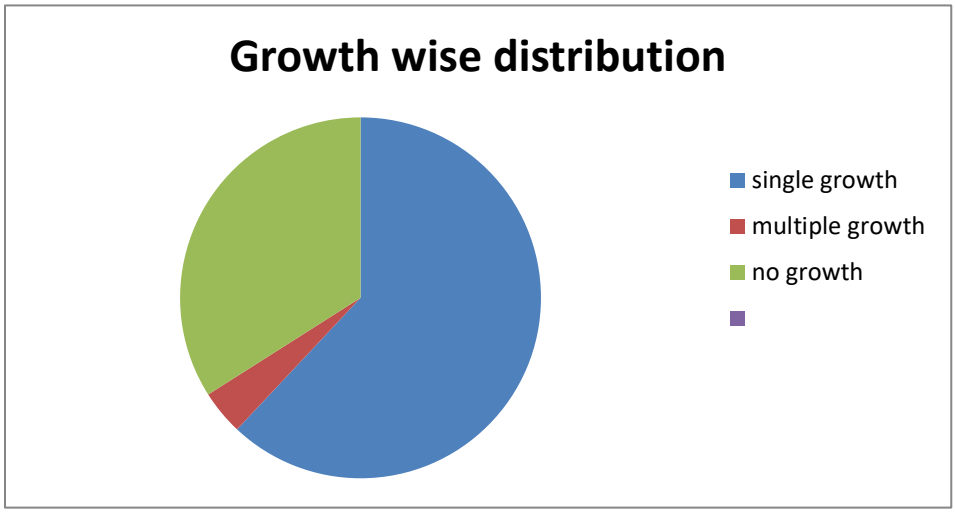

Table no.3 Growth distribution of the pus samples

Single growth	73 (62%)
Multiple growth	5 (4%)
No growth	40 (34%)

Table no.4 Gender wise distribution

Sr. No.	**Sex**	**N=118**
1	MALE	69 (58%)
2	FEMALE	49 (42%)

Among 118 samples,69(58%) male patients and 49 (42%) were female patients given in Table 4.

Table no. 5 Age wise distribution of pus samples

Sr. No	**Age**	**Total (N=118)**
1	1-20 Year	20
2	21-40 year	55
3	41-60 year	43

The age wise distribution show that the predominant age group of 21-40 years in their patients is infected with wound infection.

Table no.6 Distribution of bacterial isolates from pus samples

Positive growth	**Total isolates (N=75)**	**%**	**MDR cases (N=28)**	**MDR %**
S.aureus	35	46.7	14	50
E.coli	22	29.3	6	21.4
Pseudomonas spp.	05	6.7	0	0
Klebsiella spp.	04	5.3	2	7.14
CONS	05	6.7	4	14.28
Proteus spp	01	1.3	1	3.6
Providentia spp.	01	1.3	1	3.6
Strepto spp.	01	1.3	0	0
Citrobacter spp.	01	1.3	0	0

Figure 2: Micro-organisms MDR cases

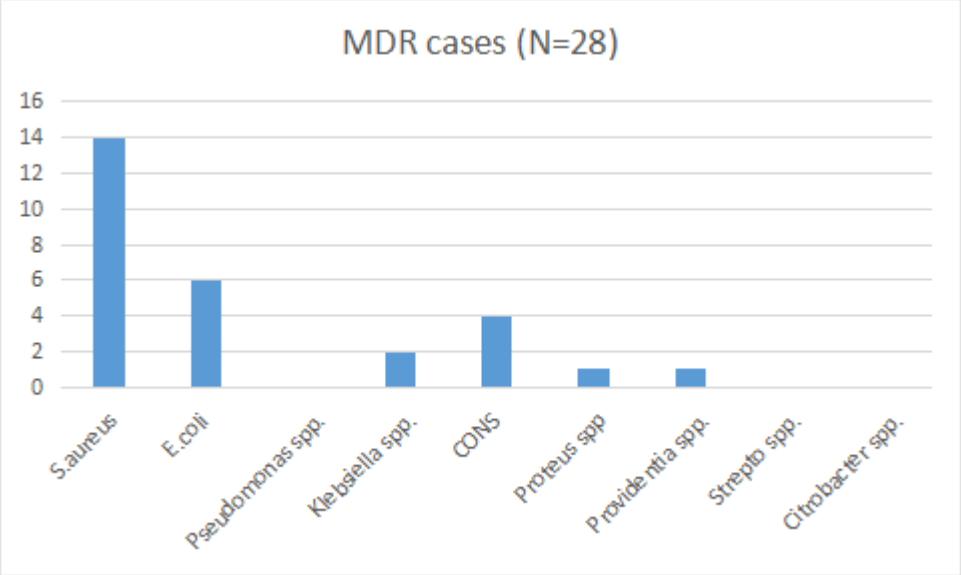

Table no. 7 Antibiotic sensitivity pattern of gram positive organism

Antibiotic	**S. aureus (N=35)**	**CONS (N=5)**	**Strepto spp. (N=1)**
Penicillin- G(P)	16 (45%)	1 (20%)	1 (1%)
CEFOXITIN(CX)	32 (91%)	5 (100%)	1 (1%)
CEFAZOLIN(CZ)	31 (88%)	5 (100%)	1 (1%)
CEFUROXIME (CXM)	29 (82%)	4 (80%)	1 (1%)
TEICOPLANIN (TEI)	34 (97%)	5 (100%)	1 (1%)
LINEZOLID(LZ)	31 (88%)	4 (80%)	1 (1%)
VANCOMYCIN (VA)	34 (97%)	5 (100%)	1 (1%)
AMOXYCLAV (AMC)	12(34%)	0	1 (1%)
CIPROFLOXACIN (CIP)	31 (88%)	5 (100%)	1 (1%)
LEVOFLOXACIN (LE)	33 (94%)	4 (80%)	1 (1%)
ERYTHROMYCIN (E)	25 (71%)	4 (80%)	1 (1%)
Co-trimoxazole (COT)	26 (74%)	5 (100%)	1 (1%)

DISCUSSION

Out of 118 clinical samples processed, 78 (66%) showed significant bacterial growth and no growth was observed in 40 (34%). Here samples were 69 (58%) from male patients and 49 (42%) from female patients. The samples were collected from patients ranging in age from 3 months to 84 years with age group 21-40 years being the most affected age group which agrees with the studies done by Parajuli k, Kumari et al [(13) (14).]

Out of 118 pus samples, 78 were culture positive with single growth (73, 62%) multiple growths (5, 4%) and no growth (40, 34%) Here total of 75 organisms were isolated in which gram-positive bacteria were 55% and gram- negative organism were 45%. A total of 75 bacterial isolates were obtained from 118 samples.S.aureus 35 (46.7%) was found to be the predominant organism followed by E.coli 22 (29.3%) Pseudomonas aeruginosa 5(6.7%) Klebsiella spp. 4(5.3%) Proteus spp 1 (1.3%) Providentia spp 1(1.3%)Strepto spp 1(1.3%) Citrobacter spp 1(1.3%). The results of this study show that Staphylococcus aureus, Pseudomonas aeruginosa .E.coli, Klebsiella spp., Proteus spp., Providencia spp. Citrobacter spp., CONS are found in pus samples.

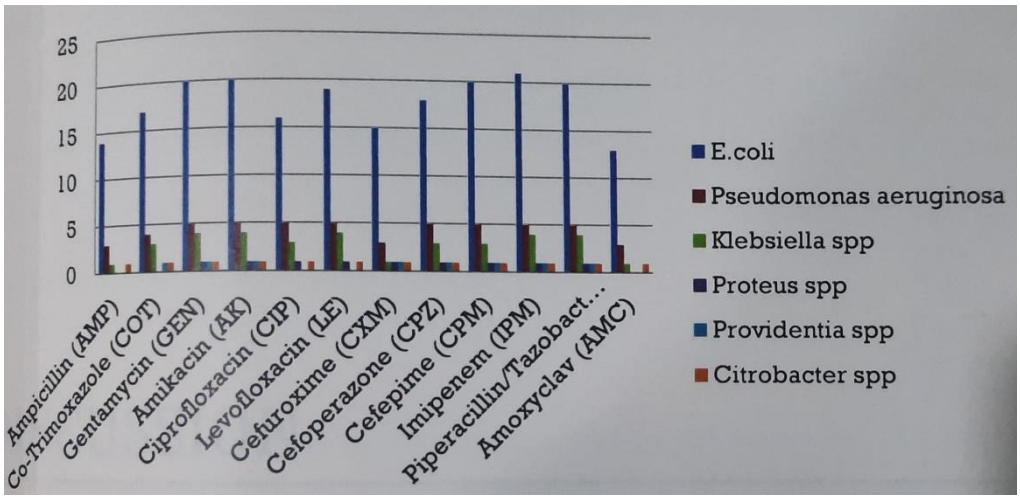

Figure 3: Antibiotic sensitivity pattern of gram negative organisms

S.aureus in this study is most sensitive to teicoplanin (97%), levofloxacin (94%) vancomycin (97%) and similar studies have reported the same findings. S.aureus was sensitive to vancomycin (100%) teicoplanin (100%) chloromphenicol(90.48%) and linezolid (100%) [(15)] S.aureus to be sensitive to vancomycin (100%) teicoplanin (100%) and linezolid (100%) [(07)] Daniel et al [(08)] reported 100 % vancomycin resistant S.aureus.[(08)]

In this study, the majority of gram-negative isolates were most sensitive to imipenem, gentamycin, amikacin, levofloxacin, cefoperazone,cefepime. This is in agreement with the study Rao et al 2014 [(16)] that gram-negative isolates were most susceptible to levofloxacin,imipenem,amikacin, and also piperacillin/tazobactam. Gram- negative isolates in this study showed better sensitivity to amikacin than gentamycin. Pseudomonas spp was highly sensitive to Gentamycin (100%) amikacin (100%) Piperacillin/Tazobactam(100%) Cefepime (100%) and levofloxacin (100%).

CONCLUSION:

In the present study, a total of 75 organisms were identified. Here 55% gram-positive organisms and 45% of gram-negative organisms were isolated in this study. The mean age group of patients affected with pus and wound infection was studied gradually between the age groups of 21-40 years. Pus and wound infections were predominantly observed in males as

compared to females. 69 males and 49 females were seen among the 118 patients included in this study.

The incidence of MDR was found to be highest in S.aureus 14(50%), followed by E.coli 6 (21.4%) CONS 4 (14.28%) proteus spp. 1 (3.6%) Providencia spp. 1 (3.6%). All the isolates were subjected to antimicrobial susceptibility test.

Though wound infection is a non-eradicable problem, preventive measures, good disinfection and universal precautions, contact isolations and hospital infection control manuals helps to minimize the incidence of wound infection. Frequent and timely consultation between the microbiologist and the clinicians on wound care also plays a major role in limiting wound infection in hospitals, similarly the antimicrobial susceptibility testing result suggests that some antibiotics should be precisely use as prophylactic. The result serves as a foundation for establishing empiric therapeutic approaches for the management of such infection in simmer medical hospital and other healthcare institution.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

ETHICS STATEMENT

This study was approved by the Institute Ethics Committee, SMIMER, Surat, India.

Data Availability

All datasets generated or analysed during this study are included in the manuscript.

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