Bacteriological Profiles of Pus with Antimicrobial Sensitivity Pattern at a Teaching Hospital in Vikarabad

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A B S T R A C T

Pyogenic infections are one of the major complications of surgery and trauma. Both aerobic and anaerobic bacteria have been implicated in these infections which commonly occur under hospital environment resulting in significant morbidity, prolonged hospitalization and huge economic burden. Hence periodical monitoring of bacterial profile and their antibiotic susceptibility pattern is important. A retrospective study was undertaken to identify the microbial profile with their antibiogram among the pus culture isolates in a teaching tertiary care hospital from January 2018 to December 2018. 171 pus culture samples processed at the microbiology lab were analyzed. The isolates from positive pus culture were identified by standard protocols and antimicrobial susceptibility patterns were identified by CLSI guidelines. Positive pus cultures were obtained in 52% (89/171). Gram positive bacteria accounted for 46.2 % (43), Gram negative bacteria 53.8% (50) and *Candida* spp 1.01% (1) with predominance of *S. aureus* 30% (28) followed by *K. pneumoniae* 18% (17) and *E. coli* 17% (16). 39.5% of *S. aureus* was MRSA and 100% sensitive to vancomycin. 88% of *E. coli* and 53% of *K. pneumoniae* were ESBL producers and 100% sensitive to meropenam.

Introduction

Pyogenic infections are one of the major complications of surgery and trauma. The factors which contribute to pyogenic infections include pre-existing illness, duration of operation, wound class and wound contamination (Pushpalatha Hanumanthappa *et al.,* 2016). These infections are characterized by local inflammation, leading to pus formation. They are generally caused by one of the pyogenic bacteria, which produce the accumulation of dead leukocytes and infectious agent commonly known as pus (Koneman *et al.,* 2005). Pyogenic infections may be endogenous or exogenous. The human skin and soft tissue infections (SSTIs) are caused by microorganisms during or after

trauma, burn injuries, and surgical procedures (Cogen *et al.,* 2008). Pyogenic infections may be polymicrobial or monomicrobial in nature (Jeffery stone and Paul Cianci*,* 1997). Both aerobic and anaerobic bacteria have been isolated from wound infections which commonly occur under hospital environment resulting in significant morbidity, prolonged hospitalization and huge economic burden (Scalise *et al.,* 2015). Most common organisms encountered are *Staphylococcus aureus, Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., *Proteus* spp., *Enterococci* spp (Krige and Beckingham, 2001).

Antibiotic resistance among bacteria is becoming an increasingly serious problem throughout the world. It is said that evolution of bacteria towards multidrug resistance is unavoidable because it represents a particular aspect of the general evolution of bacteria that is un-stoppable (Courvalin, 2005). Antibiotic resistance emerges commonly when patients are treated with empiric antimicrobial drugs. Monitoring of resistance patterns in the hospital is needed to overcome these difficulties and to improve the outcome of serious infections (El-Azizi *et al.,* 2005). During the last few decades, multidrug-resistant Gram-negative bacterial strains such as *Acinetobacter baumannii*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) were increasingly associated with pus infections under hospital settings due to extensive overuse and inadequate dose regimen of antibiotics (Iredell *et al.,* 2016).

There is a need of regular analysis of the profile and antibiogram of organisms isolated and the results need to be communicated to clinician. So, the present study was undertaken to evaluate aerobic bacteriological profile along with their susceptibility to antimicrobial agents.

The main aim and objectives of this study includes to isolate and identify pathogenic bacteria from pus samples. And also to determine the antibiotic resistance profile of the isolates. As a baseline study to formulate hospital antibiotic policy and empirical treatment.

Materials and Methods

Type of study: Retrospective study

Duration of the study: January 2018 to December 2018

Place of the study: Department of Microbiology, Mahavir Institute of Medical Sciences

Inclusion criteria

Laboratory records of patients with bacterial isolates from pus samples tested for antibiotic susceptibility during the period January 2018 - December 2018.

Exclusion criteria

1. Laboratory records with incomplete data.
2. Laboratory records with no bacterial growth.
3. Laboratory records with bacterial isolates not tested for antibiotic susceptibility.

Sample processing

In our Microbiology laboratory, all pus samples collected by sterile swabs and sterile syringe aspiration are processed by inoculation on blood agar and Mac Conkey agar. Inoculated plates and the broth are incubated at 37 ˚C overnight. Culture plates checked for the bacterial growth next day. All bacterial isolates are examined for colony characteristics, Gram staining, motility and biochemical tests. Biochemical tests employed were oxidase, catalase, nitrate, urea

hydrolysis, citrate utilization, sugar fermentation, indole production test and H_2S production on TSI agar (Collee *et al.*, 2008).

Antimicrobial susceptibility testing

In our microbiology laboratory, Antibiotic sensitivity testing was done by Kirby–Bauer disc diffusion method on Mueller-Hinton agar, as per Clinical and Laboratory Standards Institute guidelines (Bauer *et al.*, 1966; Clinical and Laboratory Standards Institute 2016). Briefly, inocula were prepared for each bacterial isolate by adjusting the turbidity to 0.5 McFarland standard and spread on Muller-Hinton agar plates. Standard ATCC strains were used as control. Antibiotic discs were procured from HiMedia, Mumbai, India.

The antibiotics that were included for testing were Amikacin (30 μg), amoxicillin-clavulanic acid (30 μg), azithromycin (30 μg), aztreonam (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefuroxime (30 μg), cefazolin (30 μg), cefoxitin (30 μg), ceftazidime-clavulanic acid (30/10 μg) ciprofloxacin (5 μg), clindamycin (2 μg), gentamicin (10 μg), linezolid (30 μg), meropenem (10 μg), Penicillin-G (10 units), piperacillin-tazobactam (100/10 μg), Trimethoprim-Sulphamethoxazole (1.25/23.75 μg) tetracycline (30 μg), Tobramycin (10 μg) and vancomycin (30 μg).

Detection of MRSA (Methicillin Resistant *Staphylococcus aureus*) and ESBL (Extended Spectrum Beta-lactamases) production was done according to CLSI guidelines. (Clinical and Laboratory Standards Institute 2016)

Statistical analysis

The data was expressed as percentages and analyzed by SPSS version 21.p value <0.5 was considered significant.

Results and Discussion

Out of 171 pus culture samples, 89 (52%) gave positive result (Fig. 1). From 89 culture positive samples, 41 (46%) were males and 48 (54%) were females (Table 1). Among the department wise distribution of positive pus culture isolates, Surgery showed maximum culture positivity 38 (43%) followed by Orthopaedics 32 (36 %) and Gynaecology 10 (11 %) (Table 2). Of the 89 positive pus culture isolates, 83 yielded monomicrobial bacterial isolates, 5 yielded polymicrobial bacterial isolates and 1 fungal isolate. So a total of 94 organisms were isolated. Among 93 bacterial isolates, Gram positive and Gram negative bacteria constituted 43 (46.2%) and 50 (53.8%) respectively.

The predominant bacterial isolate was *S. aureus* 28 (30%), followed by *K. pneumonia* 17 (18%), *E. coli* 16 (17%), *Pseudomonas aeroginosa* 12 (12%), *Streptococcus pyogenes* 8(8%), *Staphylococcus epidermidis* 6(6%), *Citrobacter* spp 3(3%), *Proteus vulgaris* 2 (2%) and *Enterococcus* sp. 1(1%).The 1 fungal isolate was *Candida non albicans* spp (1.01%) (Fig. 2). All Gram positive isolates showed 100% sensitivity to vancomycin. MRSA was found in 11 (39%) and MR CONS in 2 (33%) (Fig. 3). *S. aureus* showed a high resistance to penicillin (96%), Trimethoprim-Sulphamethoxazole (68%) and ciprofloxacin (57%) (Table 3). All Gram negative isolates were sensitive to meropenem (100%). Only 5(10%) of Gram negative isolates showed sensitivity to Trimethoprim-Sulphamethoxazole. Among Enterobacteriaceae (38/93), 25 (65.8%) (Fig. 4) isolates were resistant to 3rd generation cephalosporins. ESBL production in *K. pneumoniae* 9 (53%), *E. coli* was 14 (88%), and *Citrobacter sp.* 2 (67%). Enterobacteriaceae isolates showed a sensitivity pattern of 37 (97.3%) to piperacillin-tazobactum, 33 (86.84%) to both amikacin and tobramycin, and 31 (81.57%) to

ciprofloxacin (Table 4). The non-fermenter *P. aeroginosa* showed high sensitivity to piperacillin-tazobactum (100%), amikacin (92%), gentamicin (92%), aztreonam and ceftazidime (83% each).

In our study, age of the study group ranged from 5 to 85 years, with mean age of 45 years. Among these, 46% were males, while 54% were females. In another study conducted by Subha M. and Meerah Srinivasagam (2018), 56.62% were males and 43.38% were females.

A total of 171 specimens were received in our study, predominant isolate obtained from pus samples was *Staphylococcus aureus 28 (30%)* followed by *Klebsiella pneumoniae 17(18%).* In another study conducted Pushpalatha Hanumanthappa *et al.,* (2016) at a teaching hospital in South India, *Staphylococcus aureus* was the most frequent isolate (43.6%) followed by Klebsiella sp (17.3%). In another similar study done by Khanam *et al.,* (2018), *Staphylococcus aureus* (25%) was the most frequent isolates. Similar findings were observed among pus isolates in another study conducted by Neelima *et al* (34.3%) (2013). However, Agnihotri N et al (2004) found it to be second most common pathogen after Pseudomonas spp. Though *S. aureus* was the predominant organism, gram-positive cocci accounted for 46.2% of the total isolates, 53.8% being Gram negative bacilli (GNB). This higher prevalence of GNB in pyogenic infections has been supported by a study done by Ghosh *et al.,* (2009).

Other studies showed different distribution of bacterial isolates which may be attributed to the differences in study design, geographical location and climate conditions.

In our study, Gram-positive isolates showed maximum susceptibility to vancomycin and linezolid while Gram-negative isolates were most susceptible to meropenem, piperacillin-tazobactam, Amikacin, tobramycin and ciprofloxacin. Similar results were obtained in another study conducted by Perween *et al.,* (2018). In a study conducted by Rao *et al.,* (2014), Gram positive isolates were found most susceptible to Vancomycin (100%) followed by Levofloxacin (76.92%) and Oxacillin (73.07%). Gram Negative Bacilli were susceptible to Imipenam (80%), Aztreonam (80%), Piperacillin + Tazobactum (80%), Levofloxacin (80%).

In the present study 11 (39%) of *S. aureus* was MRSA which is similar to the finding of Mohanty *et al.,* (2004) (38.56%).Whereas Pushpalatha Hanumanthappa *et al.,* (2016) reported a higher incidence of MRSA (53.96%). All the isolates of *S. aureus* were sensitive to vancomycin as comparable with Sujatha *et al.,* (2016). The ESBL producing Enterobacteriaceae in our study was 65.8% of which *K. pneumoniae* (53%) and *E. coli* was (88%), which is higher than a recent study done by Subha and Meerah Srinivasagam (2018) whose ESBL detection rate was 42.10% of which *E. coli* were 23.61% and *K. pneumoniae* 25%. According to a study done in Nepal (Shrestha *et al.,* 2011), prevalence of ESBL was much lower at 18%. The higher prevalence of ESBL in our study and increased resistance to 3rd generation cephalosporins, β-lactam- β-lactamase inhibitors, co-trimoxazole is a matter of great concern.

Periodic monitoring of susceptibility pattern need to be carried out in each hospital settings so as to detect the actual burden of antibiotic resistance in organisms and prevent the emergence of drug resistant organisms by judicial use of antibiotics. Each hospital should take proactive steps in setting up antibiotic policy guidelines and constitute a hospital infection committee to monitor the emergence of drug resistance and should

implement standard precautions among health care personnel. In most developing countries like India, patients are able to obtain antimicrobials over the counter with or without prescription from a medical practitioner. This could be another reason for high level of resistance among the isolates in the present study (Table 5).

Table.1 Age and gender distribution of study population (n=89)

Age Group	Male	Female	Total
< 20 years	10	6	16
20 - 40	14	26	40
40 - 60	9	12	21
> 60	8	4	12
TOTAL	41	48	89

Table.2 Ward wise distribution of pus culture isolates (n= 89)

S. No	Ward	Number
1	Surgery	38 (43 %)
2	Orthopaedic	32 (36 %)
3	Gynaecology	10 (11 %)
4	ENT	5 (6 %)
5	Medicine	3 (3 %)
6	Dermatology	1 (1%)
	Total	89 (100 %)

Table.3 Antibiotic sensitivity pattern of gram positive cocci

Antibiotics	*Staphylococcus aureus(n=28)*		*Staphylococcus epidermidis(n=6)*		*Streptococcus pyogenes(n=8)*		*Enterococcus sp(n=1)*	
	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)
Azithromycin(30 µg)	22(79%)	6(21%)	4(67%)	2(33%)	5(63%)	3(37%)	1(100%)	0(0%)
Cefoxitin (30 µg)	17(61%)	11(39%)	4(67%)	2(33%)	5(63%)	3(37%)	-	-
Ciprofloxacin(5 µg)	12(43%)	16(57%)	3(50%)	3(50%)	3(38%)	5(62%)	1(100%)	0(0%)
Clindamycin (2 µg)	23(82%)	5(18%)	6(100%)	0(0%)	8(100%)	0(0%)	1(100%)	0(0%)
Tetracycline(30 µg)	24(86%)	4(14%)	5(83%)	1(17%)	6(75%)	2(25%)	1(100%)	0(0%)
Gentamycin (10 µg)	20(71%)	8(29%)	4(67%)	2(33%)	7(88%)	1(12%)	-	-
Linezolid (30 µg)	27(96%)	1(4%)	6(100%)	0(0%)	8(100%)	0(0%)	1(100%)	0(0%)
Penicillin-G(10units)	1(4%)	27(96%)	0(0%)	6(100%)	2(25%)	6(75%)	0(0%)	1(100%)
Trimethoprim-Sulphamethoxazole (1.25/23.75 µg)	9(32%)	19(68%)	1(17%)	5(83%)	2(25%)	6(75%)	-	-
Vancomycin(30 µg)	28(100%)	0(0%)	6(100%)	0(100%)	8(100%)	0(0%)	-	-

Table.4 Antibiotic sensitivity pattern of gram negative bacilli

Antibiotics	K.pneumoniae(n=17)				E.coli (n=16)				Proteus (n=2)				Citrobacter(n=3)			
	S	%	R	%	S	%	R	%	S	%	R	%	S	%	R	%
Amikacin (30 µg)	17	100%		0%	12	75%	4	25%	2	100%	0	0%	2	67%	1	33%
Amoxicillin-clavulanic acid (30 µg)	5	29%	12	71%	3	19%	13	81%	0	0%	2	100%	2	67%	1	33%
Aztreonam (30 µg)	11	65%	6	35%	14	88%	2	13%	2	100%	0	0%	2	67%	1	33%
Ceftazidime (30 µg)	8	47%	9	53%	2	13%	14	88%	2	100%	0	0%	1	33%	2	67%
Cefuroxime (30 µg)	4	24%	13	76%	5	31%	11	69%	2	100%	0	0%	0	0%	3	100%
Cefazolin (30 µg)	5	29%	12	71%	1	6%	15	94%	2	100%	0	0%	0	0%	3	100%
Cefoxitin (30 µg)	9	53%	8	47%	13	81%	3	19%	0		2	100%	1	33%	2	67%
Ceftazidime-clavulanic acid (30/10 µg)	13	76%	4	24%	10	63%	6	38%	2	100%	0	0%	2	67%	1	33%
Ciprofloxacin (5 µg)	15	88%	2	12%	13	81%	3	19%	2	100%	0	0%	1	33%	2	67%
Tobramycin(10 µg)	15	88%	2	12%	15	94%	1	6%	0		2	100%	3	100%	0	0%
Meropenem (10 µg)	17	100%	0	0%	17	100%	0	0%	2	100%	0	0%	3	100%	0	0%
Piperacillin-tazobactam (100/10 µg)	16	94%	1	6%	16	100%	0	0%	2	100%	0	0%	3	100%	0	0%
Tetracycline (30 µg)	12	71%	5	29%	3	19%	13	81%	2	100%	0	0%	1	33%	2	67%
Trimethoprim-Sulphamethoxazole (1.25/23.75 µg)	1	6%	16	94%	1	6%	15	94%	2	100%	0	0%	1	33%	2	67%

Table.5 Antibiotic sensitivity pattern of *Pseudomonas* isolates

Antibiotics	Pseudomonas (n=12)			
	S	%	R	%
Amikacin (30 µg),	11	92%	1	8%
Aztreonam (30 µg)	10	83%	2	17%
Ceftazidime (30 µg)	10	83%	2	17%
Ciprofloxacin (5 µg)	7	58%	5	42%
Gentamicin (10 µg)	11	92%	1	8%
Meropenem (10 µg)	12	100%	0	0%
Piperacillin-tazobactam (100/10 µg)	12	100%	0	0%

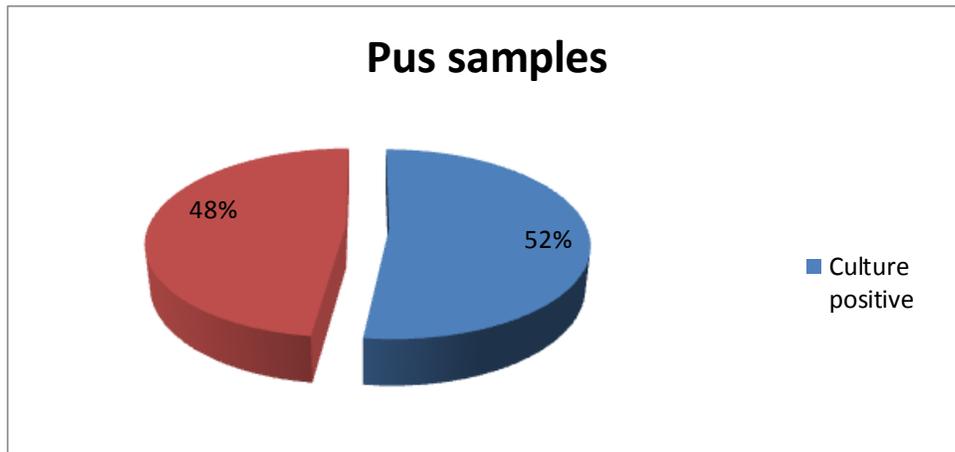**Fig.1** Pie chart showing culture positivity among pus sample

Fig.2 Distribution of aerobic bacterial isolates (n= 93) among the positive pus culture specimens
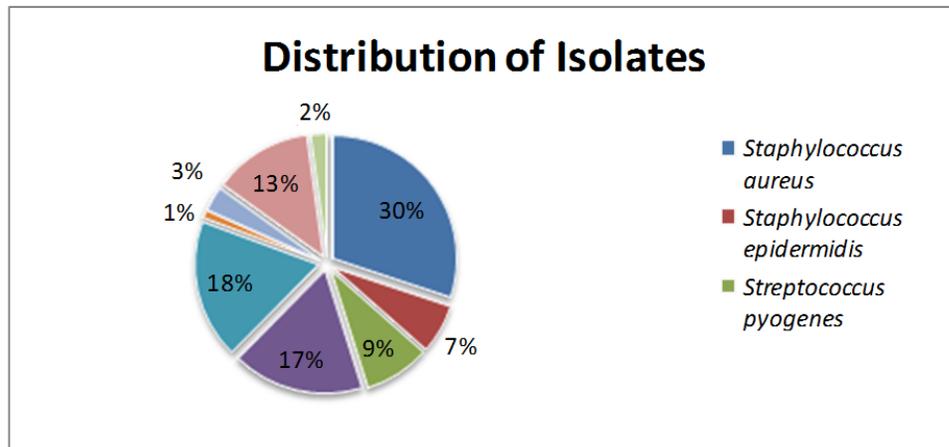

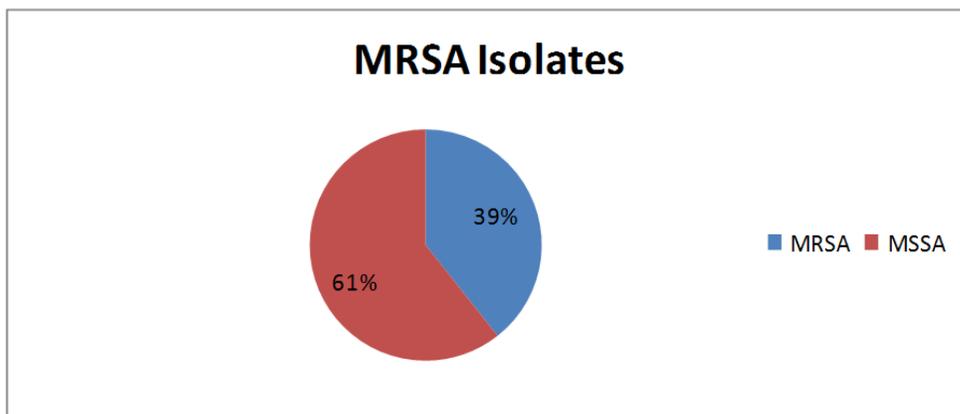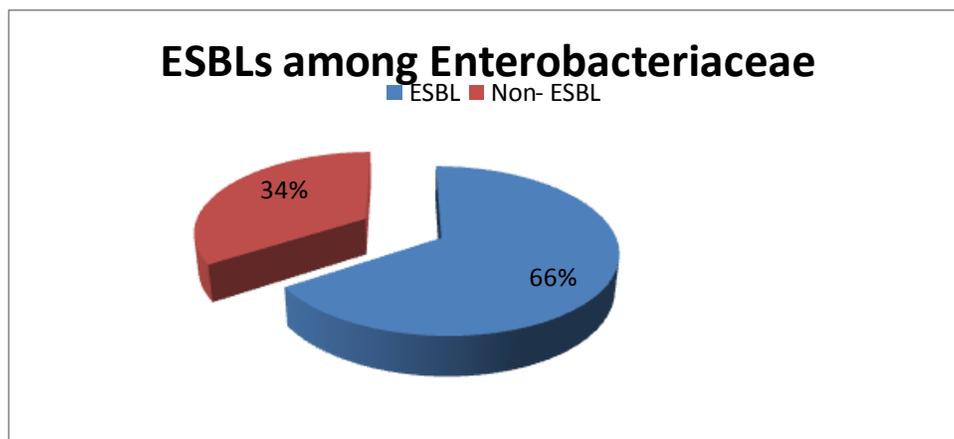**Fig.3** Showing MRSA isolates

Fig.4 Showing ESBL producers among enterobacteriaceae

It is concluded that, in the clinical care setting, pyogenic infections are still a leading cause of morbidity following surgery and trauma. The most common bacteria isolated after aerobic culture of pus was *Staphylococcus aureus*. However, Gram negative bacilli have dominated Gram positive cocci as etiological agents of pyogenic infections. Among the GNB, *Klebsiella pneumoniae* is the most common bacteria causing these infections. The bacterial isolates showed a high degree of antimicrobial resistance. Knowledge of common pathogens and their resistance status can guide clinician to choose appropriate antibiotics for empirical treatment of patients. Hence, periodic review of the bacteriological profile and antibiotic sensitivity pattern is highly essential.

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