Int.J.Curr.Microbiol.App.Sci (2016) *5*(1): 95-102

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 1(2016) pp. 95-102**
Journal homepage: http://www.ijcmas.com

Original Research Article doi: http://dx.doi.org/10.20546/ijcmas.2016.501.008

A Study on aerobic Bacteriological profile and Drug sensitivity pattern of Pus samples in a tertiary care hospital

Pushpalatha Hanumanthappa*, B. Vishalakshi and S. Krishna

Department of Microbiology, Vijayanagar Institute of Medical Sciences, Ballari, India

**Corresponding author*

Keywords

Antimicrobial susceptibility pattern, Bacteriological profile, *Klebsiella*, Methicillin Resistant *Staphylococcus aureus*,

Article Info

Accepted:
08, December 2015
Available Online:
January 2016

A B S T R A C T

The aim of study was to determine the commonly encountered pathogens in pus samples along with their antibiotic susceptibility pattern. This study was conducted from January 2014 to May 2014, in VIMS, MCH central lab. Pus samples received were processed and identification was done by standard protocols. Antibiotic susceptibility test was done by Kirby Bauer disc diffusion method. MRSA detected as per CLSI guidelines. Out of 500 pus samples received for culture and sensitivity, 280 (56%) cases yielded positive culture, 195(39%) cases remained sterile. Among the 280 culture positive pus samples, 271 yielded pure bacterial isolates and 9 yielded two organisms. Among the remaining 25 samples, 16 (3.2%) samples yielded either contaminants/ commensals, 4 (0.8%) yielded *Candida* spp and 5(1%) showed polymicrobial growth. *Staphylococcus aureus* was the most common isolate followed by *Klebsiella* spp and *E.coli*. Methicillin resistance in *S. aureus* was found to be 53.96% (68). Among the Gram positive isolates, vancomycin and Ampicillin were the most susceptible drugs whereas among the Gram negative isolates the most susceptible drugs were aminoglycosides. Majority of the wounds were infected with a single organism so, proper management of pus infection with the appropriate antibiotic must be implanted and emphasized to minimize emergence of drug resistant bacteria.

Introduction

Pyogenic infection is one of the major complications of surgery and trauma. The factors which contribute to pyogenic infections include preexisting illness, length of operation, wound class and wound contamination (Ramesh Rao *et al*., 2013).

These infections may be endogenous or exogenous (Koneman *et al*., 2005) or it may be polymicrobial or monomicrobial in nature (Jeffery stone *et al*, 1997).

The pathogens isolated from infections

differ depending on the underlying problem, location and type of surgical procedure. (Ramesh Rao *et al*., 2013). Most common organisms encountered are *Staphylococcus aureus, Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., *Proteus* spp., *Enterococci* spp (Krige J.E.J., and Beckingham J.I, 2001) *Enterobacter*, *Proteus* spp, *Candida* and *Acinetobacter* spp (Tayfour MA *et al*., 2005).

For prevention & cure of pyogenic infections, antibiotics play a key role. To select an appropriate antibiotic needs knowledge of the potential microbial pathogen, its pathophysiological role in the infectious process and an understanding of the pharmacology and pharmacokinetics of the intended antibiotics. (Kelwin W.S,1999)

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.

Materials and Methods

Study Setting

The study population was of patients irrespective of age and sex either admitted to different wards in hospital or visiting the out-patient department.

Study Period

Record based observational study was conducted at VIMS, MCH Ballari over a five months period from January 2014 to may 2014.

Exclusion Criteria

Diphtheroids, environmental bacillus species and > =3 agents (contaminants) in the samples.

A total number of 500 pus samples received for aerobic culture and sensitivity from different wards & OPDs in Microbiology Central laboratory of VIMS MCH Hospital, Ballari during a period from January to May 2014 were included in the study. Informed consent was taken from the patient and ethical clearance was obtained from the institute.

Taking aseptic precautions, lesions were cleaned with sterile normal saline. With proper care, avoiding contamination by the normal flora of skin or mucus surface, the pus was aspirated or exudate collected. The specimens were transported in sterile, leak-proof containers to the lab immediately or if there was a delay, stored at 4^0C; those with refrigeration of more than 24 hours were not processed.

The pus samples were subjected for gram stain to look for pus cells and organisms. Specimens were inoculated on blood agar and MacConkey agar plates and incubated overnight at 37^0C. Pathogens were identified by conventional biochemical methods according to standard microbiological techniques (Collec JG *et al*., 1996).

The antimicrobial susceptibility testing was done by Kirby Bauer's Disk Diffusion method and interpreted as per Clinical Laboratory Standard Institution (CLSI) guidelines (CLSI, 2012).

Standard antibiotics like, ampicillin (10 mcg), vancomycin (30 mcg), ceftriaxone (30 mcg), cefotaxime (30 mcg), ceftazidime (30 mcg), ciprofloxacin (5mcg), co-trimoxazole (1.25/23.75 mcg), gentamycin (10 mcg), amikacin (30 mcg), clindamycin (2mcg) and erythromycin (15mcg), Cefoxitin (30mcg)

were tested (Himedia, Mumbai, India) (Betty A. Forbes *et al*., 2007)

Detection of Methicillin Resistance

The methicillin resistance in *Staphylococcus* spp. was tested by cefoxitin disc (30 µg) as documented in Clinical and Laboratory Standard Institute (CLSI 2012). (Cefoxitin disc diffusion of ≤21 mm for *S. aureus*). The positive culture reports were analysed and percentages and proportions were calculated.

Results and Discussion

Out of 500 pus samples received for culture and sensitivity in the Microbiology central laboratory, 280 (56%) cases yielded positive culture, 195(39%) cases remained sterile even after 48hrs incubation. Among the 280 culture positive pus samples, 271 yielded pure bacterial isolates and 9 yielded two organisms; so a total number of 289 organisms were isolated out of 500 pus samples. Among the remaining 25 samples, 16 (3.2%) samples yielded either contaminants/ commensals, 4 (0.8%) yielded *Candida* spp and 5(1%) showed polymicrobial growth (>= 3 organisms) as shown in table 1.

Table.1 Rank order of pus samples isolates

Rank order	Number of samples
Total	500
Aerobic growth with one organism	271
Aerobic growth with two organism	9
No growth	195
Commensals/ contaminants	16
Polymicrobial (>=3 org)	5
Candida spp	4

Of the 289 isolates, there were 157 (54.32%) gram negative bacilli and 132 (45.67%) gram positive cocci. Most common organism isolated was *Staphylococcus aureus* 126(43.6%) followed by *Klebsiella spp 50 (17.3%).* Other isolates included were, *E.coli 41*(14.18%), *Pseudomonas spp 34*(11.76%), *Citrobacter spp 16* (5.54%), *Proteus* spp. 10 (3.46%) *Enterococcus spp 6* (2.07%), *Enterobacter spp 3* (1.03%),*Burkholderia spp 1 (0.35%), Acinetobacter1 (0.35%), and other nonfermenting gram negative bacilli1 (0.35%).*As shown in flow chart 1.

Flow chart 1: Flow chart showing 289 aerobic bacterial isolates of pus samples

Table 2 Antibiotic sensitivity pattern of gram positive cocci

Among the *S. aureus*, vancomycin, Ampicillin and gentamicin were the most susceptible drugs with 87.3%, 59.5% and 59.5% respectively. And *S. aureus* showed least sensitivity to Cefotaxime, Erythromycin, Co-trimoxazole with 54.7%, 53.9% and 52.3% respectively.

Enterococcus spp most sensitive to ampicillin, ceftriaxone and erythromycin with 66.6%, 50 % and50% respectively and least sensitive to 3rd generation cephalosporines like cefotaxime and ceftazidime with 83.3% and 83.3% respectively.

Gram negative isolates are most sensitive to aminoglycosides and Ceftazidime and least sensitive to Ciprofloxacin,and Co-trimoxazole. The observations of our study coincide with the various studies across the country. The predominance of mono-microbial infections observed in our study is substantiated by a study done by Basu S *et al* (Basu S *et al*., 2009).

Table.2 Antibiotic Sensitivity Pattern of Gram Positive Cocci

Antibiotics(µg/disc)	S. aureus (126)		Enterococcus spp (6)	
	S (%)	R (%)	S (%)	R (%)
Ampicillin (30)	75 (59.5)	51 (40.4)	4 (66.6)	2 (33.3)
Amikacin(30)	96 (76.1)	30 (23.8)	2 (33.3)	4 (66.6)
Co-trimoxazole (1.25/23.75)	60 (47.6)	66 (52.3)	-	-
Ciprofloxacin(5)	70 (55.5)	56 (44.4)	2 (33.3)	4 (66.6)
Ceftriaxone (30)	66 (52.3)	60 (47.6)	3 (50)	3 (50)
Cefotaxime(30)	57 (45.2)	69 (54.7)	1 (16.6)	5 (83.3)
Ceftazidime(30)	61 (48.4)	65 (51.5)	1 (16.6)	5 (83.3)
Cefoxitin (30)	58 (46.1)	68 (53.9)	-	-
Clindamycin (2)	72 (57.1)	54 (42.8)	3 (50)	3 (50)
Erythromycin (15)	58 (46.1)	68 (53.9)	3 (50)	3 (50)
Gentamycin (10)	75 (59.5)	51 (40.4)	-	-
Vancomycin (30)	110 (87.3)	16 (12.6)	-	-

Flow chart.1 Flow Chart Showing 289 Aerobic Bacterial Isolates of Pus Samples

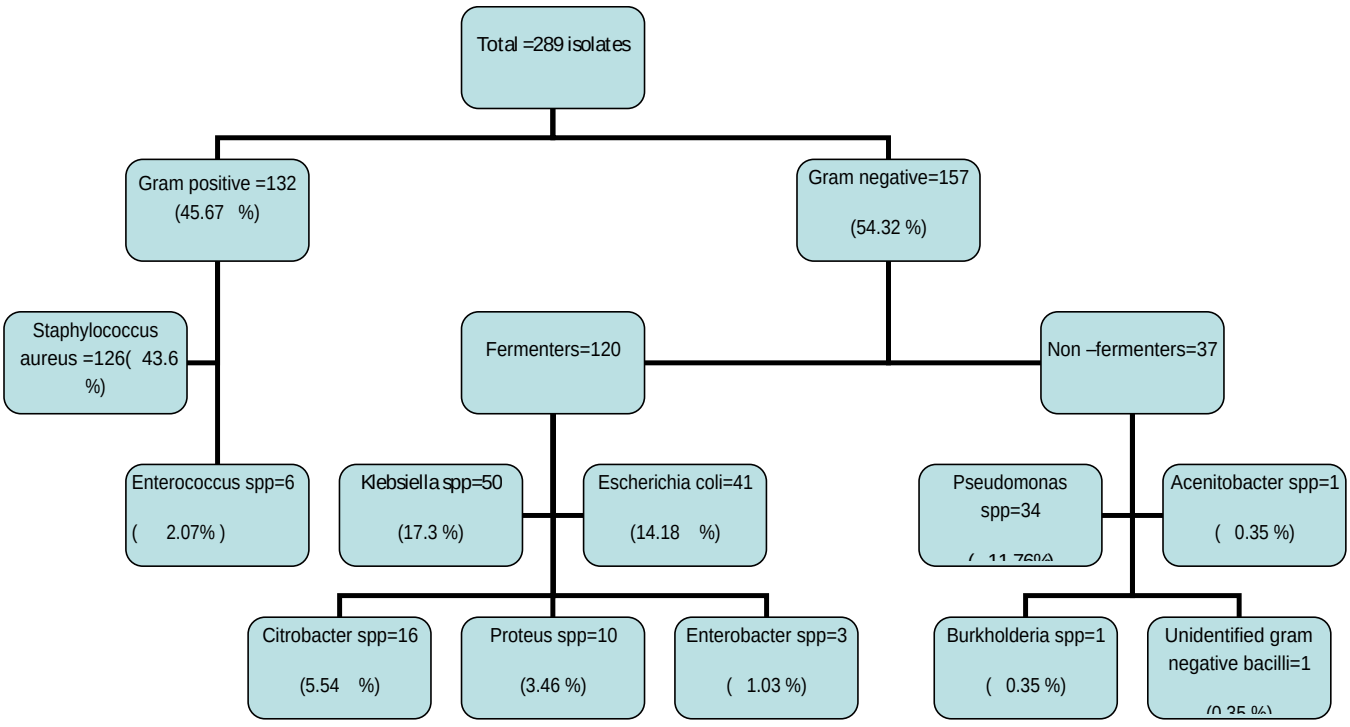

Table.3 Antibiotic Sensitivity Pattern of Gram Negative Bacilli

Sl. No.	Antibiotics (µg/disc)	*Klebsiella* (50)		*E. coli* (41)		*Citrobacter* spp (16)		*Proteus* spp (10)		*Enterobacter* (3)		*Pseudomonas* (34)	
		S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
1.	Ampicillin (30)	20 (40)	30 (60)	15 (36.5)	26 (63.5)	9 (56.2)	7 (43.7)	7 (70)	3 (30)	0 (0)	3 (100)	-	-
2.	Amikacin (30)	37 (74)	13 (26)	25 (60.9)	16 (39.1)	9 (56.2)	7 (43.7)	7 (70)	3 (30)	2 (66.66)	1 (33.33)	19 (55.9)	15 (44.11)
3.	Co-trimoxazole (1.25/23.75)	-	-	11 (26.8)	30 (73.1)	-	-	-	-	3 (100)	0 (0)	-	-
4.	Ciprofloxacin (5)	19 (38)	31 (62)	18 (43.9)	23 (56.1)	6 (37.5)	10 (62.5)	4 (40)	6 (60)	0 (0)	3 (100)	14 (41.17)	20 (58.8)
5.	Ceftriaxone (30)	24 (48)	26 (52)	12 (29.2)	29 (70.7)	5 (31.2)	11 (68.7)	3 (30)	7 (70)	0 (0)	3 (100)	16 (47.05)	18 (52.9)
6.	Cefotaxime (30)	32 (64)	18 (36)	13 (31.7)	28 (68.2)	4 (25)	12 (75)	4 (40)	6 (60)	0 (0)	3 (100)	16 (47.05)	18 (52.9)
7.	Ceftazidime (30)	15 (30)	35 (70)	9 (21.9)	32 (74.1)	6 (37.5)	10 (62.5)	6 (60)	4 (40)	1 (33.33)	2 (66.66)	14 (41.17)	20 (58.8)
8.	Gentamycin (10)	38 (76)	12 (24)	28 (68.2)	13 (31.7)	7 (43.7)	9 (56.2)	6 (60)	4 (40)	2 (66.66)	1 (33.33)	10 (29.4)	24 (70.5)

In the present study, *S. aureus* 126 (43.6%) is the most common pathogen isolated. Similar studies conducted showed Neelima *et al* (34.3%) (Neelima *et al*., 2013), Tapan at Navodaya Medical college, Raichur who also reported *S.aureus* (27.5%), at Kathmandu model hospital (41.31%) (Shrestha B, Basnet RB, 2009), another study was conducted in TUTH showed (57.7%) (Kensekar P *et al*., 2003). This is comparable with that of Tiwari P, Kaur S (Tiwari P, Kaur S, 2010), Lee CY *et al* (Lee CY *et al*., 2009). However, Agnihotri N *et al* (Agnihotri N *et al*., 2004) found it to be second most common pathogen after *Pseudomonas* spp. while *Klebsiella*, *Pseudomonas* and *Escherichia coli* were the three leading Gram-negative isolates compared to that of Lee CY *et al* (Lee CY *et al*., 2009).

In the present study, the prevalence of MRSA is 53.96% which is higher than that reported from Nagpur (19.56%) (Tahnkiwale SS *et al*., 2002) and Vellore (24%) (Pulimood TB *et al*, 1996), in India. However, it is comparable to that in Mohanty *et al* (2004) about 38.56%, United States and certain European countries where methicillin resistance was detected in 32.4% to 44.4% *S.aureus* isolates. (Jones ME *et al*., 2003)

Among the *S. aureus*, vancomycin, Ampicillin and gentamicin were the most sensitive drugs and showed least sensitivity to Cefotaxime, Erythromycin, Co-trimoxazole. *Enterococcus* spp showed most sensitive to ampicillin, ceftriaxone and erythromycin and least sensitive to 3rd generation cephalosporines. Gram negative isolates are most sensitive to aminoglycosides and Ceftazidime and least sensitive to Ciprofloxacin,and Co-trimoxazole. However, tests for identification of ESBL production were not performed, thus leaving further scope of evaluation.

The susceptibility pattern obtained in our study suggests that the most common organisms are gram-positive cocci, notably *S. aureus*, many of them are methicillin-resistant. Therefore, empirical antibiotic treatment should be primarily directed against this pathogen. Use of single drug therapy with cephalosporins, aminoglycosides and fluoroquinolones need to be guided by the antibiogram. Hospitals should screen for MRSA among their staff and treat those who harbor them.

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 work precautions among health care personnel.

Our study concludes that, majority of the pus samples yielded mono-microbial growth. *S. aureus* being the commonest pathogen; the role of gram negative bacilli cannot be undermined. Clinician should initiate the empirical treatment based on bacteriological and antibiogram as baseline data. The present study provides one-time information about the antibiogram which is not sufficient, as the periodic review of the bacteriological profile and antibiotic sensitivity pattern is highly essential.

Acknowledgement

The authors wish to thank the residents of the Department for providing the relevant

data about the patients included in the study and in helping us to collect samples for culture.

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How to cite this article:

Pushpalatha Hanumanthappa, B. Vishalakshi and Krishna S. 2016. A Study on aerobic Bacteriological profile and Drug sensitivity pattern of Pus samples in a tertiary care hospital. *Int.J.Curr.Microbiol.App.Sci.* 5(1):95-102. doi: http://dx.doi.org/10.20546/ijcmas.2016.501.008