Int.J.Curr.Microbiol.App.Sci (2017) *6*(10): 1405-1413

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 10 (2017) pp. 1405-1413**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.610.166

Bacteriological Profile and the Antibiotic Susceptibility Pattern of Microorganisms Isolated from Pus/Wound Swab Isolates in Patients Attending a Tertiary Care Hospital in South India

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Keywords

Pus culture, Sensitivity, Resistance, MRSA, ESBL.

Article Info

Accepted:
14 September 2017
Available Online:
10 October 2017

A B S T R A C T

The aim of the study was to determine the commonly isolated aerobic microorganisms found in pus samples and their antibiotic sensitivity pattern. This study was conducted in the Government Medical College Hospital, Thoothukudi from January to December 2016. Pus samples received from various departments are processed by standard protocols. Antibiotic susceptibility was done by Kirby Bauer disc diffusion method. Among 1575 pus samples received for culture and sensitivity in the microbiology laboratory, 1126 (71.49%) samples yielded positive culture and there was no growth in 449 (28.51%) samples. Among the 1126 culture positive cases 665 (59.06%) were male and 461 (40.94%) were female and the male: female ratio is 1.44.*Klebsiella species* was predominantly isolated 253 (22.5 %) followed by *Staphylococcus aureus* was 208 (18.5%), *Escherichia coli* was 194 (17.2%), *Pseudomonas aeruginosa* was 178 (15.8%), *Proteus species* was 166 (14.7%), *Citrobacter species* was 33 (2.9%), *Acinetobacter species* was 30(2.7%), *Coagulase Negative Staphylococcal species* 55 (4.9%), *Enterococci species* was 9 (0.8%). Gram Negative bacteria were mostly susceptible to Amikacin, Piperacillin Tazobactum, Ceftazidime-clavulanic acid, Cefaperazone sulbactum and Imipenem whereas Gram positive organisms show 100% sensitivity to Vancomycin and linezolid. In this study, about 54% of the isolates were ESBL producers. The incidence of ESBL isolates were high in *Escherichia coli* (60%) followed by *Klebsiella* species (56%), Pseudomonas aeruginosa (51%), *Acinetobacter* sp (50%), *Citrobacter* species (52%), *Proteus* species (49%) among Gram Negative Bacilli. MRSA isolated were 41% of *Staphylococcus aureus* and 49% of *Coagulase negative Staphylococcal species.* The study gives the bacterial profile in the wound infections and its sensitivity pattern which is very important for clinicians to start empirical treatment for patients, while culture reports are awaited. This study suggests reserving carbapenem drugs for critically ill patients as the antibiotic pipeline is already dry.

Introduction

Pyogenic infections are associated with involvement of production of pus and bacteria. This may involve synergistic combination of aerobic and anaerobic species of bacteria which may be present either in singles or in mixed combinations which require antibiotic therapy. The probability of wound infections largely depends on local

wound conditions, microbial burden and the host defense conditions. Effective treatment of wound infections depends upon proper understanding of causative pathogen, pathophysiology of the infectious process and pharmacology of the therapeutic agents. Multidrug resistant organisms continue to be important cause of Hospital acquired infection and pose a therapeutic challenge (Chaudhari *et al.,* 2007; Gupta and Datta, 2007).

Because of irrational use of antibiotics, virulent strains adapt to the environment and it is a concern to the health care services. The antibiotic pipeline has become dry and it is the need of the hour to reserve antibiotics like carbapenems to multidrug resistant organisms (Amreliwala *et al.,* 2015).

Continuous monitoring of bacterial profile from pus samples and its sensitivity would highlight variations in the resistance pattern of the organisms. The present study was done to monitor the aerobic bacterial profile and their antibiogram in pus samples in this region which is relevant for the clinicians in treating the patients empirically.

Materials and Methods

Study design

This study was conducted using a total number of 1575 pus/ wound samples received for aerobic culture and sensitivity from various Departments in the Microbiology laboratory of Government Medical College Hospital, Thoothukudi during a period from January 2016 to December 2016.

Inclusion criteria

All pus samples/ wound swab collected in sterile swabs and containers were included in the study.

Exclusion criteria

Pus samples received in unsterile containers were rejected.

Identification and antibiotic susceptibility testing

Pus samples were received in two sterile swab sticks or in sterile container. First swab stick is used for Gram staining and second one is used for culture. Received pus samples were processed on blood agar, MacConkey agar, Nutrient agar media and incubated at 37^{o}C under aerobic condition and the organisms are identified by Gram stain, motility testing, biochemical reactions (Catalase, Oxidase, Indole, citrate utilisation, urease test, Triple sugar Iron test, Mannitol salt agar) using standard microbiological methods (Collee *et al.,* 1996; Koneman, 2006). For antibiotic sensitivity, bacterial suspensions of the isolate is prepared and matched with 0.5 McFarland standard for lawn culture. The antimicrobial susceptibility testing were done by modified Kirby Bauer disc diffusion method and interpreted by CLSI guidelines 2017.Standard antibiotics like penicillin G (10units), amoxicillin-clavulanicacid (20/10mcg), amikacin (30mcg), Gentamicin (10mcg), Tobramycin (10mcg), Erythromycin (15mcg), ciprofloxacin (5mcg), ceftriaxone (30mcg), ceftazidime (30mcg), ceftazidime clavulanic acid (30/10mcg), Cefaperazone sulbactum (75/10mcg), cotrimoxazole (1.25/23.75mcg), piperacillin/tazobactum (100/10mcg), Vancomycin (30mcg), Linezolid (30mcg) and cefoxitin (30mcg) for screening MRSA were obtained from Himedia, Mumbai India.

Screening of MRSA (Methicillin resistant *Staphylococcus aureus*)

Cefoxitin 30 µg disc is considered as surrogate marker for detection of methicillin resistant *Staphylococcus aureus* isolates. Zone

of inhibition of less than 21mm is considered as Methicillin resistant (CLSI guidelines 2017).

Screening of ESBL (Extended Spectrum Beta Lactamase)

A disc of cefotaxime (30 µg) and ceftazidime (30 µg) were kept placed on the agar surface in addition to the other antimicrobial agents. The plate was incubated at 37°C overnight. The zone diameter of <=27mm and<=22mm for Cefotaxime and Ceftazidime respectively indicated that the isolate may produce ESBL.

Phenotypic ESBL confirmatory method

By using the phenotypic disc diffusion test (CDDT), all the organisms were screened for ESBL production. Organism was considered as ESBL producer, if the ceftazidime - clavulanic acid zone size is more than or equal to 5 mm with comparison to ceftazidime disc zone size (CLSI guidelines 2017).

Quality control

Staphylococcus aureus ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as quality controls strains. Results obtained were analysed by percentages using MS Excel 2010.

Results and Discussion

Out of 1575 pus samples received for culture and sensitivity in the microbiology laboratory, 1126 (71.49%) samples yielded positive culture and there was no growth in 449 (28.51%) samples (Figure 1). Among the 1126 culture positive samples, 900 yielded pure bacterial isolates and 226 yielded mixed infection. Among the 1126 culture positive cases 665 (59.06%) were male and 471 (41.8%) were female and the male: female ratio is 1.44 (Table 1).

Among the 1126 culture positive samples, *Klebsiella species* was predominant bacterial isolate 253 (22.5 %) followed by *Staphylococcus aureus* was 208 (18.5%), *Escherichia coli* was 194 (17.2%), *Pseudomonas aeruginosa* was 178 (15.8%), Proteus species was 166 (14.7%), *Citrobacter species* was 33 (2.9%), *Acinetobacter species* was 30 (2.7%), *Coagulase Negative Staphylococcus species* was 55 (4.9 %), *Enterococci species* was 9 (0.8%).

Polymicrobial infection with two or more organisms occurred in 20.1% cases. *Staphylococcus aureus* was most commonly isolated among the Gram positive cocci. *Klebsiella sp* was most commonly isolated among Enterobacteriaceae followed by *E. coli*, *Proteus* sp. (Figure 2) (Table 2).

Antibiotic susceptibility pattern of Organisms isolated

Klebsiella species isolated were 68% sensitive to amikacin, 48% sensitive to Ceftazidime, 44% sensitive to Cetriaxone, 42% sensitive to Ceftazidime-clavulanic acid, 56% sensitive to Cefaperazone sulbactum and 83% sensitive to Imipenem. Antibiotic resistance of *Klebsiella species* is very high in third generation cephalosporins (Table 3).

E. coli isolated were susceptible to species isolated were 75% sensitive to amikacin,44% sensitive to Ceftazidime, 40% sensitive to Cetriaxone, 64% sensitive to Cefaperazone sulbactum 56% sensitive to Ceftazidime-clavulanic acid and 89% sensitive to Imipenem (Table 3).

Proteus species isolated were susceptible to species isolated were 49% sensitive to amikacin, 31% sensitive to Ceftazidime, 51%

sensitive to Cetriaxone, 72% sensitive to Cefaperazone sulbactum, 67% sensitive to Ceftazidime-clavulanic acid and 80% sensitive to Imipenem (Table 3).

Pseudomonas aeruginosa is sensitive to 74% sensitive to amikacin, 62% sensitive to Cefaperazone sulbactum, 79% sensitive to Piperacillin tazobactum, 44% sensitive to Ceftazidime, 40% sensitive to Cetriaxone, 47% sensitive to Ciprofloxacin, 54% sensitive to Ceftazidime-clavulanic acid and 87% sensitive to Imipenem (Table 4).

Acinetobacter sp is sensitive to 83% sensitive to amikacin, 60% sensitive to Cefaperazone sulbactum, 73% sensitive to Piperacillin tazobactum, 50% sensitive to Ceftazidime, 47% sensitive to Cetriaxone, 53% sensitive to Ciprofloxacin, 57% sensitive to Ceftazidime-clavulanic acid and 87% sensitive to Imipenem (Table 4).

In this study, about 54% of the isolates were ESBL producers. The incidence of ESBL isolates were high in *Escherichia coli* (60%) followed by *Klebsiella* species (56%), *Citrobacter* species (52%), *Proteus* species (49%) among Enterobacteriaceae. Among non-fermenting gram negative bacilli ESBL was detected in *Pseudomonas aeruginosa (51%)* followed by *Acinetobacter* species (50%) (Table 6).

Fig.1 Pie chart showing culture positivity among pus sample

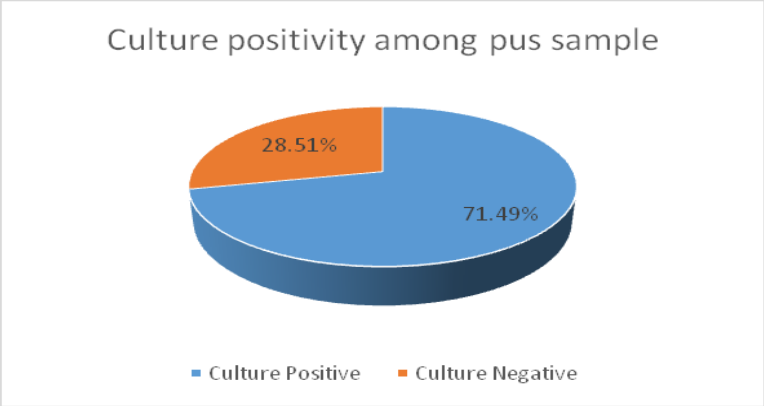
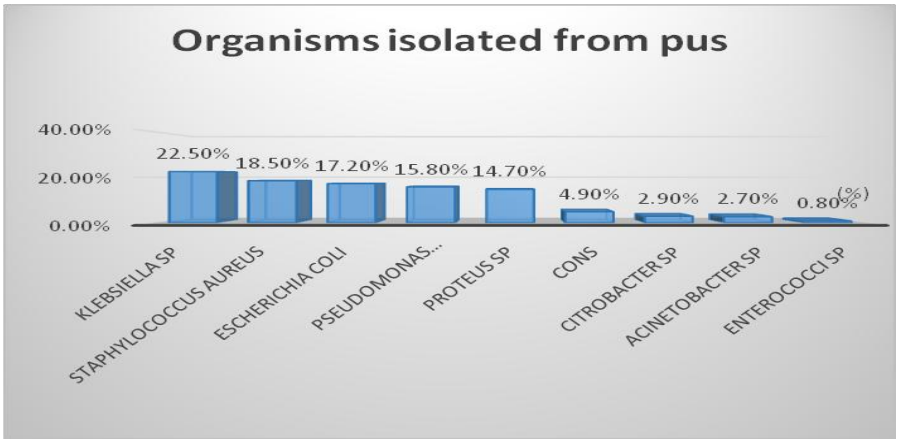


Fig.2 Bar Graph showing distribution of organisms isolated from pus samples



*CONS – Coagulase negative Staphylococcal species

Table.1 Sex wise distribution of culture positive pus samples

Sex	Culture positive (n=1126)
Male	665 (59.06%)
Female	461 (40.94%)

Table.2 Aerobic bacteria (n=1126) isolated from pus samples

S.No.	Organism	Number (%)
1.	Klebsiella sp	253 (22.5%)
2.	*Staphylococcus aureus*	208 (18.5%)
3.	Escherichia coli	194 (17.2%)
4.	*Pseudomonas aeruginosa*	178 (15.8%)
5.	Proteus sp	166 (14.7%)
6.	CONS	55 (4.9%)
7.	Citrobacter sp	33 (2.9 %)
8.	Acinetobacter sp	30 (2.7%),
9.	Enterococci sp	9 (0.8 %)
Total		1126

Table.3 Pattern of antibiotic sensitivity and resistance for Enterobacteriaceae

Name of Antibiotics	*Klebsiella* sp (n=253)		*E. coli* (n=194)		*Proteus* sp (n=166)		*Citrobacter* sp (n=33)	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	42(17%)	211(83%)	47(24%)	147(76%)	52(31%)	114(69%)	12(36%)	21(64%)
Amikacin	172(68%)	81(32%)	146(75%)	48(25%)	82(49%)	84(51%)	24(72%)	6(18%)
Gentamycin	133(52%)	120(48%)	60(31%)	134(69%)	23(14 %)	143(86%)	22 (67%)	8(24%)
Ciprofloxacin	110 (43%)	143(57%)	98(51%)	96(49%)	34(20 %)	132(80%)	17(51%)	16(49%)
Ceftazidime	123(48%)	130(52%)	86(44%)	108(56%)	52(31%)	114(69%)	15(45%)	18(55%)
Ceftriaxone	112(44%)	141(56%)	77(40%)	117(60%)	85(51%)	81(49%)	16(48%)	17(52%)
Ceftazidime-clavulanic acid	105(42%)	148(58%)	108(56%)	86(44%)	112(67%)	54(33%)	11(33%)	22(66%)
Cefaperazone sulbactum	142(56%)	111(44%)	124(64%)	70(36%)	120(72%)	46(28%)	19(58%)	14(42%)
Imipenem	209 (83%)	44(17%)	172(89%)	22(11%)	133(80%)	33(20%)	26(79%)	7(21%)

Table.4 Pattern of antibiotic sensitivity and resistance for Non fermenters

Name of Antibiotics	*Pseudomonas aeruginosa* (n=178)		*Acinetobacter* spp (n=30)	
	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	32 (18%)	146(82%)	5(17%)	25(83%)
Amikacin	131(74 %)	47(26%)	25(83%)	5(17%)
Gentamicin	69(39 %)	109(61%)	17(57%)	13(43%)
Tobramycin	138(78%)	40(22%)	25(83%)	5(17%)
Ciprofloxacin	83(47%)	95(53%)	16(53%)	14(47%)
Cotrimoxazole	0	0	0	0
Ceftazidime	88(49%)	90(51%)	15(50%)	15(50%)
Ceftriaxone	72(40%)	106(60%)	14(47%)	16(53%)
Ceftazidime-clavulanic acid	96 (54%)	82(46%)	17(57%)	13(43%)
Cefaperazone sulbactum	110(62%)	68(38%)	18(60%)	12(40%)
Piperacillin-Tazobactum	141 (79%)	37(21%)	22(73%)	8(27%)
Imipenem	155(87%)	23(13%)	26(87%)	4(13%)

Table.5 Pattern of antibiotic sensitivity and resistance for Gram positive cocci

Name of Antibiotics	*Staphylococcus aureus* (n=208)		CONS (n=55)		*Enterococci* (n=9)	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	48(26%)	140(75%)	18(33%)	37(67%)	3(33%)	6(67%)
Clindamycin	113(60%)	75(40%)	14(25%)	41(75%)	0	0
Ciprofloxacin	35(19%)	153(81%)	18(33%)	37(67%)	0	0
Erythromycin	94(50%)	94(50%)	34(62%)	21(38%)	2(22%)	7(78%)
Amikacin	124(66%))	64(34%)	40(73%)	15(27%)	0	0
Gentamycin(HLG)	0	0	0	0	7(78%)	2(22%)
Cotrimoxazole	62(33%)	126(67%)	13(24%)	42(76%)	0	0
Cefoxitin	124(66%)	64(34%)	28(51%)	27(49%)	0	0
Amoxicillin-clavulanic acid	76(41%)	112(59%)	30(54%)	25(46%)	0	0
Vancomycin	208(100%)	0	55(100%)	0	9(100%)	0
Linezolid	208(100%)	0	55(100%)	0	9(100%)	0

Table.6 ESBL isolates in Gram negative bacilli

Organisms	ESBL n (%)	Non ESBL n (%)	Total
Klebsiella sp	141(56%)	112(44%)	253
E. coli	117(60%)	77(40%)	194
Proteus sp	81(49%)	85(51%)	166
Citrobacter sp	17(52%)	16(48%)	33
Pseudomonas sp	90(51%)	88(49%)	178
Acinetobacter	15(50%)	15(50%)	30
Total	461 (54%)	393(46%)	854

Gram positive organisms mainly *Staphylococcus aureus* isolated were 100% sensitive to Vancomycin and Linezolid, 66% sensitive to amikacin, 41% sensitive amoxicillin-clavulanic acid, 50% sensitive to Erythromycin and 60% sensitive to clindamycin. *Methicillin resistant Staphylococcus aureus* isolates were detected by cefoxitin disc diffusion method. 41% of *Staphylococcus aureus* and 49% of *Coagulase negative Staphylococcus species* were resistant to Cefoxitin (Table 5).

This study shows male preponderance (59.06%) as compared to female (41.80%). It closely corroborates with the study by Raghav Rao *et al.,* (2014) which shows highest occurrence in males (58.82%).This was similar to studies of siddiqui *et al.,* (2002), Bhatt and Vassikar (2010), Pappu *et al.,* (2011), Rajeshwar Rao *et al.,* (2014).

Gram Negative bacteria were most predominantly isolated from pus culture samples compared to Gram Positive bacteria. This was similar to studies by Aziza zafar (2008), Ghosh *et al.,* (2009), Zubair etal., (2011), Sarath babu *et al.*,(2012), Rajeswar Rao *et al.*,(2014), Ravichitra *et al.,* (2014), Nirmala and Rajesh (2017).

Klebsiella sp was the most commonly isolated organisms 253(23%). This was observed in other studies like Sarath babu *et al.,* (2012), Rajeswar Rao *et al.,* (2014), Kritu Panta *et al.,* (2013), Ravichitra *et al.,* (2014), Vijeta *et al.,*

(2015) and Nirmala and Rajesh (2017). In contrast, some studies Karia *et al.,* (2013) highlighted *Staphylococcus aureus* was isolated in increased frequency whereas in other study by Ramesh Kannan *et al.,* (2014) *E. coli* was isolated more commonly.

In this study, about 54% of the isolates were ESBL producers. While in India, the ESBL prevalence ranges from 60 to 70% (Menon *et al.,* 2007) (Dalela, 2012). There is high incidence of ESBL isolates in *Escherichia coli(60%)*followed by *Klebsiella* species (56%), *Citrobacter* species (52%),*Proteus* species (49%) among gram negative bacilli under family Enterobacteriaceae similar to study of Basavaraj *et al.,* (2011).

In this study 41% of Methicillin resistant *Staphylococcus aureus* is isolated which correlates with a study by Arora *et al.,* (2010) from North India where the prevalence of MRSA was 46 %. In a study conducted by Indian network for Surveillance of Antimicrobial Resistance (INSAR) group, India shows the overall MRSA prevalence was 42 per cent in 2008 and 40 per cent in 2009 (INSAR group, India 2013).

The organisms most commonly isolated from the pus culture samples were *Klebsiella species* followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. MRSA screening by cefoxitin disc diffusion method reveals 41% of *Staphylococcus aureus* and 49% of *Coagulase negative Staphylococcus specie*s. Among Gram Negative bacteria's, 54% of the isolates were ESBL producers. Among Gram Negative bacteria, all the organisms were highly sensitive to Aminoglycosides, Cefaperazone sulbactum, Piperacillin Tazobactum, Imipenem and Gram Positive bacteria's were all 100% sensitive to Vancomycin and Linezolid. This study did not address the detection of Amp C prevalence in our set up which remains to be the limitation and gives a scope to focus in the future research.

This study gives an outline of antibiotic susceptibility of clinical isolates which will help in formulating the local antibiotic policy for the hospital. This study suggests that beta lactum and its betalactamase combination like Piperacillin tazobactum or Cefaperazone sulbactum and aminoglycosides like Amikacin instead of choosing carbapenemsin treating hemodynamically stable patients. Hence this study would help in adhering to appropriate empirical therapy for the specific organisms and also prevent emergence and spread of resistant isolates.

Due to non-availability of newer antibiotics for the treatment purpose it is necessary to monitor resistance pattern among the clinical samples so that right choice of drug can reduce the burden to physicians during the patient care. This study emphasis to reserve carbapenems for only seriously ill patients.

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How to cite this article:

Nithya Gomatheswari, S. and Jeyamurugan, T. 2017. Bacteriological Profile and the Antibiotic Susceptibility Pattern of Microorganisms Isolated from Pus/Wound Swab Isolates in Patients Attending a Tertiary Care Hospital in South India. *Int.J.Curr.Microbiol.App.Sci.* 6(10): 1405-1413. doi: https://doi.org/10.20546/ijcmas.2017.610.166