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SPECTRUM OF AEROBIC BACTERIAL PATHOGEN ISOLATED FROM PUS SAMPLES AND THEIR ANTIBIOTIC SENSITIVITY PATTERN IN A TERTIARY CARE HOSPITAL

Microbiology

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ABSTRACT

Background: Wound infection continues to be a significant problem in clinical practice where empirical treatment of infection is required while waiting for culture report to prevent emergence of resistance.

Objectives: To study the spectrum of aerobic bacterial pathogen isolated from pus samples and to determine the antimicrobial sensitivity pattern of the bacteria isolated.

Method: The samples of pusreceived in Clinical Microbiology Laboratory were processed during the study period to isolate and identify bacterial pathogens as per standard bacteriologicaltechniques. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method as per CLSI guidelines 2017.

Result: Out of the total of 1474pus samples processed, 21.98% samples were culture positive. Majority of culture positive samples were from IPD patients.Predominance of gram negative bacteria (77.4%) was seen as compared to gram positive bacteria (22.60%) both in IPD and OPD samples. High level of resistance to various antimicrobials was seen among the clinical isolates.

Conclusion: Emergence of antibiotic resistant strains in pus sample is a matter of concern. Therefore, knowledge of the spectrum of microorganisms causing pus discharge and its susceptibility pattern is required and this data may contribute to an effective management of cases of wound infection.

KEYWORDS

Wound infection, aerobic bacteria,pus, antibiotic susceptibility

INTRODUCTION

Infectious disease isthe one of the common cause of morbidity and mortality worldwide.[1] Pyogenic infection is caused by pyogenic bacteria and is characterized with pus formation, which is produced because of the accumulation of dead leukocytes and infectious agent.[2]These infections may be exogenous or endogenous. A break in the skin can provide entry of the surface bacteria into the wound which thereby start multiplying locally. Eventually, accumulation of these cells produces pus which is a thick whitish liquid.[2,3]

Many wound infections and abscesses are poly-microbial, particularly those that result from fecalspillage, bedsores, and infections in diabetic patients.[2] There is considerable dispute about the value of identifying and testing the antimicrobial susceptibility of multiple isolates, problems of sampling make it difficult to ensure that all pathogenic species have beenrecovered.

The rapidemergence and spread of multidrug-resistant bacteriais consideredas threat to the public health worldwide due to the limited available treatment options.[4,5]This study has been carried out with the objective to know the spectrum of pyogenic bacteria isolated from pus samplesand to determine their antibiotic susceptibility so that empiric therapy can be carried out for better patient outcome.

MATERIAL AND METHODS

This prospective study was carried out in Department of Microbiology, Subharti Medical College and associated Chattrapati Shivaji Subharti Hospital (CSSH), Meerut, over a period of one year (Oct. 2016 to Sept.2017). Pus samples collected with all aseptic precaution; either with sterile disposable cotton swabs and/or frank pus /aspirates in syringe were transported in to the clinical microbiology laboratory for isolationand identification of bacterial pathogen.

The samples were inoculated on to Blood agar (BA) and MacConkey agar (MA) plates. Simultaneously the samples were also inoculated in Brain-Heart Infusion (BHI) broth for enrichment. All culture plates and BHI broth were incubated at 37℃ for 24 to 48 hours. Any growth in culture media,the colony morphology of the bacterial pathogen/ pathogens were observed and documented. The isolated bacterial pathogen were identified using standard bacteriological methods, [6,7] which included colony morphology, Gram stain and battery of biochemical tests such as catalase, oxidase, indole, urease, citrate, triple iron sugar, sugars, OF, KingsA, KingsB, amino acids,coagulase, bile asculineboth for Gram positive and Gram negative bacteria as per the finding of Gram stain.

If at 24 or 48 hours there was no growth on the plates but there was turbidity on BHI broth, the sub-culture from the broth was done on the culture plates and incubated aerobically.[6] Any growth observed now was also identified as mentioned above.

The antibiotic sensitivitytesting was performed by Kirby Bauer's disc diffusion method on Muller Hinton Agar(MHA) plate and interpreted as per CLSI guidelines.[8]

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa*ATCC 27853,*Staphylococcus aureus*ATCC 25923 was used for quality control.

RESULT

A total of 1474pus samples were processed during the study period , out of which 324 (21.98%) pus samples were culture positive and 1150 (78.01%) showedno growth.Among the culture positive samples (n=324), majority were samples received from IPD patients (n= 292) and (n=32) were from OPDpatients.

Male predominance (64.04%& 59.37%) was seen as compared to female (27.05% & 31.25%)both in IPD&OPD culture positive samples respectively.Maximum cases were from Surgery 113(38.6%) followed by Orthopaedics 43(14.7%),SICU 35 (11.9%), Emergency ward 22 (7.53%). The samples received from ENT, Labour room and burn units were less in number.

Majority 240 (82.2 %) of culture positive cases from IPD, had mono-microbial etiology as compared to poly-microbial aetiology 52(17.80 %). There was predominance of GNB (n=226) in both monomicrobial

and polymicrobial group as compared to GPCs (n=66). Similarly, among the culture positive cases from OPD,majority 26 (81.25%) had mono-microbial etiology as compared to 6 (18.75%) which had poly-microbial aetiology. In OPD cases also there was predominance of GNB (n=22) in both monomicrobial and polymicrobial group as compared to GPCs (n=10).

In IPD samples out of the total 292 isolates, *E.coli* 83(28.4%) was the predominant followed by K*lebsiella spp.* 44 (15.06%), *Pseudomonas spp*. 40 (13.6%) *Acinetobacter spp.*20(6.84%), *Proteus spp*. 23(7.87%), *Citrobacterspp.* 9 (3.02%), *Morganella spp.* 5 (1.71%), *Burkholderia spp.* 2(0.68%), *CONS* 35 (11.98%),*Staphylococcus aureus* 25(8.56%) and *Enterococcus spp*. 6(2.05 %). [Table 1] In OPD Out of the total 32 isolates, *Pseudomonas spp*. 9 (28.1%) was the predominant followed by *E.coli* 5 (15.6%) *Klebsiellaspp.* 4(12.5%), *Acinetobacter spp.* 1(3.12%), *Proteus spp.* 2(6.25%), *Citrobacterspp.* 1(3.12%), *CONS*8(25%), *S.aureus*2(6.25%).[Table 1]

Table 1: Spectrum of aerobic bacterial pathogen isolated from pus samples

Organism (IPD)	No.	Rate (%)	Organism (OPD)	No.	Rate (%)
GNB			**GNB**		
E.coli	83	28.4	*E.coli*	5	15.6
Klebsiella spp.	44	15.06	*Klebsiella spp.*	4	12.5
Pseudomonas spp.	40	13.6	*Acinetobacter spp.*	1	3.12
Acinetobacterspp	20	6.84	*Proteus spp.*	2	6.25
Proteus spp.	23	7.87	*Citrobacter spp.*	1	3.12
Citrobacter spp.	9	3.02	*Pseudomonas spp.*	9	28.1
Morganell spp.	5	1.71			
Burkholderia spp.	2	0.68			
GPC			**GPC**		
CONS	35	11.98	*CONS*	8	25
Staphylococcus aureus	25	8.56	*Staphylococcus aureus*	2	6.25
Enterpcoccus spp.	6	2.05			

Looking at the antibiotic sensitivity pattern of the clinically isolated pathogens it was found that majority of the isolates were resistant to various groups of antimicrobial agents both in IPD and OPD patients as well as among GNBs and GPCs [Table 2, 3&4].

Table 2: Sensitivity of Gram negative bacteria isolated from IPD samples to various antibiotics (%)

GNB	*E.coli* (n=83)	*Klebsiella spp* (n=44)	*Acinetobacter spp.* (n=44)	*Proteus spp.* (n=23)	*Citrobacter* spp. (n=9)	*Morganella spp.* (n=5)	*Pseudomonas spp.* (n=40)	*Bulkhulderia Spp.* (n=2)
AMP	1	0	nt	34	0	0	nt	nt
PI	0	0	20	39	0	0	25	50
AMC	0	0	nt	39	0	0	nt	nt
A/S	6	9	30	52	22	20	nt	nt
PIT	0	27	25	0	0	0	47	50
TE	20	6	nt	IR	33	0	nt	nt
COT	12	11	30	26	33	40	nt	nt
CIP	6	2	30	39	22	60	20	nt
CFM	2	6	nt	43	11	0	nt	nt
CAZ	2	6	25	43	11	0	35	50
CTR	2	6	25	43	11	0	nt	nt
AT	2	6	nt	43	11	0	30	50
CPM	4	9	25	43	11	100	53	50
GEN	63	27	25	82	55	60	30	50
AK	66	27	25	82	55	60	30	50
TOB	64	25	30	82	55	60	30	50
C	74	31	Nt	69	55	20	nt	nt
ETP	70	40	Nt	95	66	80	nt	nt
MRP	70	40	50	95	66	80	55	50
IPM	70	43	50	91	66	80	62	100
CL	100	100	100	IR	100	IR	100	100

nt=not tested, IR=Intrinsically resistance

AMP- Ampicillin, PI- Piperacillin AMC- amoxicillin/clavulanic acid, A/S- ampicillin-sulbactum, PIT- itperacillin/tazobactam, TE- Tetracycline, COT- Cotrimoxazole, CIP-Ciprofloxacin, CFM- Cefixime, CAZ- Ceftazime, CTR- Ceftriaxone, AT- Aztreonam , CPM- Cefepime, GEN- Gentamicin, AK- Amikacin, TOB- Tobramycin, C- Chloramphenicol, ETP- Ertapenem, MRP- Meropenem, IPM- Imipenem, CL- Colistin.

Table 3: Sensitivity of Gram positive cocci isolated from IPD samples to various antibiotics (%)

GPC	*Staphylococcus aureus* (n=25)	*CONS* (n=35)	*Enterococcus spp.* (n=6)
Penicillin	0	0	16
Ampicillin	nt	nt	16
Erythromycin	56	28	16
Clindamycine	76	28	16
Doxycycline	nt	nt	Nt
Co-trimoxazole	12	28	Nt
Tetracycline	84	57	Nt
Ciprofloxacin	20	31	Nt
Moxifloxacin	36	37	Nt
Chloramphenicol	88	77	16
Gentamicin	88	85	Nt
Linezolid	100	71	100
Vancomycin	100	71	100
Teicoplanin	nt	nt	100
HLG	nt	nt	33
HLS	nt	nt	33

nt=not testedHLS= High level Gentamicin, HLS= High level Streptomycin

Table 4: Sensitivity of Gram negative and Gram positive bacteria isolated from OPD samples to various antibiotics (%)

GNB	E.coli n =5	Klebsiellaspp n=4	Acinetobacter Spp. n=1	Proteus spp. n=2	Citrobacter Spp. n=1	Pseudomonas Spp. n=9	GPC	Staphylococcu aureus n=2	CONS n=8
AMP	0	25	Nt	50	0	22	P	0	25
PI	0	50	0	50	0	22	E	50	25
AMC	0	25	Nt	100	0	22	CD	100	50
A/S	0	25	0	100	0	22	COT	0	37
PIT	0	25	0	50	0	66	TE	100	62
TE	0	25	Nt	IR	0	22	CIP	0	25

COT	0	25	0	50	0	22	MO	50	12
CIP	0	25	0	50	1	22	C	100	75
CFM	0	25	Nt	100	0	22	GEN	100	62
CAZ	0	25	25	100	0	33	LZ	100	100
CTR	0	25	0	100	0	22	VA	100	100
AT	0	25	Nt	100	0	33			
CPM	0	25	0	100	0	22			
GEN	100	0	0	100	0	33			
AK	100	0	0	100	100	33			
TOB	100	0	0	100	100	33			
C	100	75	Nt	100	0	nt			
ETP	100	25	Nt	100	100	nt			
MRP	100	25	0	100	100	100			
IPM	100	25	100	100	100	100			
CL	100	100	100	IR	100	100			

nt=not tested,IR=Intrinsically resistance

AMP- Ampicillin, PI- Piperacillin AMC- amoxicillin/clavulanic acid, A/S- ampicillin-sulbactum, PIT- itperacillin/tazobactam, TE- Tetracycline, COT- Cotrimoxazole, CIP-Ciprofloxacin, CFM- Cefixime, CAZ- Ceftazime, CTR- Ceftriaxone, AT- Aztreonam , CPM- Cefepime, GEN- Gentamicin, AK- Amikacin, TOB- Tobramycin, C- Chloramphenicol, ETP- Ertapenem, MRP- Meropenem, IPM- Imipenem, CL- Colistin, P- Penicillin, E- Erythromycin, CD- Clindamycine, COT- Co-trimoxazole, TE- Tetracycline, CIP- Ciprofloxacin, MO- Moxifloxacin, LZ- Linezolid, VA- Vancomycin

DISCUSSION

A total of 1474pus samples were processed during the study period, out of which only21.98% pus samples were culture positive.On the contraryRao*et. al*[9]showed a high (89.47%)culture positivity rate and only 10.53% cases to be culture negative.Comparatively high rate (78.01%)of culture negativity in our study may be due to following reasons; firstly our centre being a tertiary care hospital patients usually come to us after seeking medical advice from local doctors and incomplete course of antibiotics, which might have led to sterile cultures in clinically suspected cases.Secondly, these infections may have been caused by anaerobic bacteria which were not looked forwhich is also one of the limitation of our study.

Among the culture positive samples,majority of the pus samples were received from IPD patients (90.12%) ,as compared to OPD (9.87%). On gender wise distribution there was male predominance (64.04% & 59.37%)from IPD & OPD samples respectively. Our data wascomparable to a study carried out by Pankaj*et al*.where they also showed male predominance. The relatively higher cases in males may be due to greater participation of males in outdoor activitythus more prone to wound infections.[10]

In the present study we received maximum number of pus samples from Surgery department 113(38.6%) followed by Orthopaedics 43(14.7%)and Emergency ward 22(7.53%). Similarly, Roopa*et al* in their study reported maximum number of pus samples from surgery department 198 (67.57%) followed by medicine department 46 (15.69%).[11] Higher number of samples from surgery department has been observed in almost all studies done on pus cultures probably because maximum of pus and wound discharge cases present to surgery department. We received lesser number of samples from ENT 11(3.76%) and Medicine department 10 (3.42%).

The culture positive samples (IPD and OPD) hadpredominantly mono-microbial aetiology as compared to poly-microbial aetiology in our study which is in complete agreement with a study carried out by Pankaj*et.al.,* where they also reported growth of single organism in 52.23%and multiple growths in 7.0%.[10]

There was predominance of Gram negative bacteria (77.4%) isolated from pus samples compared to gram positive bacteria in our study and *E.coli* (28.4%) was the predominant GNB isolated followed by *Klebsiella spp.* (15.06%) and CONS(11.98%) was the predominant GPC isolated followed *Staphylococcus aureus*(8.56%).[Table 1]This finding is in completeagreement to several earlier studies.Kannan*et. al*, also found *E.coli* (61%) as the most common organism in their study.[12] On the contrary study by Chauhan*et al*,reported *S.aureus*as the commonest organism isolated in 46% followed by *Pseudomonas* spp.and *E.coli* , 20.5% each.[13]

Pseudomonas spp. 9(28.1%) was the predominant GNBs isolated in OPD samples followed by *E.coli* 5(15.6%),*Klebsiellaspp* 4(12.5%),*Acinetobacter spp.* 1(3.12%), *Proteus spp.* 2(6.25%), *Citrobacterspp* 1(3.12%)and CONS8(25%) was the predominant GPC followed by *S. aureus*2(6.25%).[Table 1]

Looking at the profile of the antibiotic sensitivity pattern of the isolated pathogens it was found that majority of the isolates were resistant to various groups of antimicrobial agents [Table 2,3&4].Resistance was also observed towards carbapenems (meropenem, imipenem, ertapenem), which is a matter of great concern. Such high level of resistance tocarbapenemsis an alarm for the judicious use of these drugs.However, allour clinical isolates of GNBs were 100%sus ceptible to colistin and GPCs were susceptible to vancomycin, linezolid, teicoplanin.

We reported a very high level of resistance to penicillin in both IPD and OPD samples. Smililarly, Bindu*et.al.*also reported similar findings in their study.[14]

To conclude, high level of resistance to various antimicrobial agents was observed in cases of pus and the emergence of antibiotic resistant strains has led to treatment failure. Therefore, knowledge of the spectrum of microorganisms causing pus dischargeand its susceptibility pattern is required and this data may contribute to an effective management of cases of wound infection.

CONFLICT OF INTREST

Nil

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