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A Study of Various Isolates from Pus Sample with Their Antibiogram from Jln Hospital, Ajmer

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Abstract: *This study was conducted in the Department of Microbiology, J. L. N. Medical College, Ajmer (Raj.) for a period of 6 months. A total of randomly 100 pus samples received in the bacteriology section of microbiology department from the various wards of J.L.N Hospital to identify the common aerobic bacterial isolates and to study their antimicrobial susceptibility. Out of 100 culture, 48 (48%) were gram negative and 25 (25%) were gram positive. Mixed growths were seen in 20 (20%) samples and no growth in (7%) cases. The most common organism isolated was Klebsiella (28%) followed by Pseudomonas aeruginosa 20 (20%), Coagulase negative staphylococcus 10 (10%), Staphylococcus aureus 8 (8%), Proteus mirabilis 5 (5%), Enterococcus faecalis 4 (4%), E.coli 3 (3%), Citrobacter 2(2%) and Streptococcus pyogens in 3 (3%) of case. It was observed from that gram positive organisms were sensitive to Gentamicin, Amoxyclav, Linezolid and Vancomycin but resistant to Ampicilline and Cotrimoxazole. For Ciprofloxacin and Erythromycin sensitivity was moderate. Gram negative organisms were sensitive to Amikacin, Imipenem and moderately sensitive to Tetracycline, Ciprofloxacin, Cefotaxime and amoxyclave.*

Keywords: *Wound Samples, Gram negative isolates, Gram positive isolates, antimicrobial sensitivity*

I. Introduction:

Pyogenic infection is characterized by several local inflammation, usually with pus formation, generally caused by one of the pyogenic bacteria, which can produce the accumulation of dead leukocytes and infectious agent commonly known as pus (Koneman etal., 2005). The most common pus producing bacteria are Staphylococcus aureus (S. aureus), Klebsiella spp., Pseudomonas, Escherichia coli and Streptococci in which S. aureus is the most common bacteria that produces pus (A.R. Kumar, 2013).Microorganisms are the concealed enemies to the mankind and cause a very profound damage in human body as well as other living organism. The agents, which have the capacity to kill the microbes or arrest the multiplication, are called the antimicrobial agents or drugs. There are a lot of antimicrobial drugs of which some are discovered or established (Suganya etal., 2012). Various studies across the globe have been consistent enough to show a predictable bacterial profile in the pyogenic wound infections. This makes an important observation for a clinician who intends to start empirical treatment to his patients. The present study was designed to evaluate the profile of aerobic pyogenic bacteria along with their susceptibility to antimicrobial agents. (Rao etal., 2014). The microbiology laboratory plays a central role in the decision to choose a particular antimicrobial agent over others. First, causative organism is identified and isolated when the patients' specimens are sent to the microbiology laboratory. Once the microbial species causing the disease have been identified, a rational choice of the class of antibiotics likely to work in on the patient can be made (Goodman & Gillman's, 2010).

II. Material And Methods:

A total of 100 pus sample were collected from various wards of J.L.N hospitals, Ajmer. Wound samples were collected using sterile cotton swabs contained in the small screw capped bottles, a firmly stopper tube or syringe or a sealed capillary tube and it was labeled with the patients name, age, sex etc.

Processing of Sample:

Two sterile swab sticks were used to collect the pus samples. 1st swab stick was used for gram staining and IInd swab stick was used for culture. Direct smear with gram stain were screened for the presence of inflammatory cells and type of microbial flora. IInd swab was inoculated on MacConkey agar, Blood agar and Brain Heart Infusion Broth. It was incubated at 37°C for 24-48 hrs. Observe the growth, if there was no growth on MA & BA but BHI was turbid, and then subculture was done on MA & BA. The colonial morphology and identification was done by standard procedures (Collee etal. 1996). Biochemical tests applied were standard catalase test, citrate utilization, coagulase, oxidase, methyl red, Voges-Proskauer, indole production, motility, carbohydrate fermentation test using glucose, sucrose, maltose and lactose. Characterization and identification of the isolates was done using the methods of Cowan and Steel (1985), Mathur et al. (2006) and Senthilkumar et al. (2012).

Antibiogram Testing

The antibiogram testing was done as per as CLSI guidelines using modified Kirbey-Bauer method. Few colonies from the culture plate were inoculated into 2ml of peptone water. Incubated at 37^0C for 2 hr. Turbidity was compared to that of 0.5 Mc Farland standards. A cotton swab was immersed and rotated in this inoculums, the swab was then pressed to the side of the tube so as to remove excess inoculums. It was then used for carpet streaking on Muller Hinton agar plate. The required antibiotic discs were then placed aseptically on this medium using sterile forcep. The plate was then incubated 24 hr at 37^0C. Next day the zone size was recorded and repotted as sensitive or resistant by comparing the zone size to the Kirby-bauer chart. If the organism were not sensitive to any of the drug, then a second line of drug is put up using the same procedures as above.

Antimicrobial susceptibility testing of isolates was performed by standard Kirby Bauer disc diffusion methods according to CLSI protocol. Depending on the isolate, antibiotic discs were selected from among the following: Co-trimaxazol (25µg), Erythromycin (15µg) , Gentamicin (10µg),Ciprofloxacin (5µg), Oxacillin(1µg),Amoxyclav (30 µg), Linazolid (30 µg),Vancomycin (30 µg),Tetracycline (30µg),Cefotaxime(30µg),Amikacin(30µg),Amoxyclav(30 µg),Ceftazidime (30 µg),Imipenem (10 µg), Pipercilline (100 µg).

III. Results:

A total of hundred pus samples were received in microbiology laboratory from June 2014- Dec 2014. Maximum number of cases falls in the age group 0-10 years (22%) and 21-30 years (19%) and sample received from burn ward was 50% followed by children ward 15%, MICU 12%, plastic surgery 10%, Male medical 8% and Female medical 5%. In our present study males were more affected 55 (55%) than Females 45 (45%). Out of 100 cultures smears 48% were gram negative and 25% were gram positive. Mixed growth was seen in 20% samples and no growth in 7% cases. Single organism was isolated in 83 samples (83%) of the total cases studied. While 20 (20%) cases yielded mixed growth. In 7 (7%) of the cases the culture remained sterile. Following tables are showing results:

Incidence of the monomicrobial isolates in the present study:

S. No.	Organisms	No. of organisms (n=100)	Percentage (%) (n=100)
1	Klebsiella sp.	28	28%
2	Pseudomonas aeruginosa	20	20%
3	CONS	10	10%
4	Staphylococcus aureus	8	8%
5	Proteus mirabilis	5	5%
6	Enterococcus faecalis	4	4%
7	E.coli	3	3%
8	Citrobacter	2	2%
9	Streptococcus pyogens	3	3%
10	Mixed infection	10	10%
11	No Growth	7	7%
	Total	**100**	**100%**

Incidence of Polymicrobial isolates:

S. No.	Mixed isolates	No. of cases	Percentage (%)
1	Klebsiella pneumoniae + Pseudomonas aeruginosa	5	50%
2	Staphylococcus aureus + Pseudomonas aeruginosa	3	30%
3	Citrobacter freundii + Klebsiella pneumoniae	2	20%
	Total		**100%**

Antibiotic Sensitivity Pattern of Gram Positive Organism (Monomicrobial Isolates):

S. No.	Organism	No.	Amp		Amc		CIP		COT		GEN		E		LZ		OX		V	
			R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
1	CONS	10	20	80	0	100	20	80	20	80	0	100	0	100	0	100	0	100	0	100
2	S. aureus	8	100	0	37.25	62.5	25	75	75	25	37.5	62.5	25	75	12.5	87.5	75	25	0	100
3	E. faecalis	4	50	50	0	100	50	50	100	0	0	100	25	75	0	100	0	100	0	100
4	Step. Pyogens	3	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100

It was observed from above study that gram positive organisms were sensitive to Gentamicin, Amoxyclav, Linezolid and Vancomycin but resistant to Ampicilline and Cotrimaxazole. For Ciprofloxacin and Erythromycin sensitivity was moderate.

Antibiotic Sensitivity Pattern of Gram Negative Isolates (Monomicrobial):

S. No.	Organism	No.	AK		Amc		CIP		CTX		CAZ		IMP		TE	
			R	S	R	S	R	S	R	S	R	S	R	S	R	S
1	Klebsiella sp.	28	28.57	71.42	46.42	53.57	39.28	60.71	28.57	71.42	32.14	67.85	0	100	46.42	53.57
2	Proteus mirabilis	5	0	100	40	60	60	40	40	60	20	80	0	100	80	20
3	E. coli	3	0	100	33.33	66.66	33.33	66.66	33.33	66.66	33.33	66.66	0	100	33.33	66.66
4	Citrobacter frundi	2	0	100	50	50	50	50	50	50	50	50	0	100	50	50

Gram negative organisms were sensitive to Amikacin and Imepienem and for Tetracycline, Ciprofloxacin, Cefotaxime and amoxyclave , all are moderately sensitive.

Antibiotic Sensitivity Pattern for Pseudomonas:

Organism	No.	AK		AMC		CIP		CTX		CAZ		IMP		PI		TE	
		R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Ps	20	0	100	40	60	40	60	40	60	35	65	0	100	25	75	50	50

All Pseudomonas aeruginosa were 100% sensitive to Imipenem and Amikacin followed by Piperacilline (75%), Ceftazidime (65%), Amoxyclave, Ciprofloxacin and cefotaxime (60%) and Tetracycline (50%).

Antibiotic Sensitivity Patteren of Polymicrobial Isolates:

S. No.	Organism	No.	AK		AMC		CIP		CTX		CAZ		IMP		TE		LZ		OX		V		AMP		COT		GEN		E		PI	
			R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
1	K.pneumoniae+P. aeruginosa	5	-	S	R	-	-	S	R	-	-	S	-	S	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			-	S	R	-	-	S	R	-	R	-	-	S	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S
2	S. aureus+ P. aeruginosa	3	-	-	-	S	R	-	-	-	-	-	-	-	-	-	-	S	R	-	-	S	R	-	R	-	-	S	R	-	-	-
			-	S	-	S	-	S	-	S	-	S	-	S	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S
3	C. frundii+ K. Pneumoniae	2	-	S	R	-	-	S	R	-	R	-	-	S	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			-	S	R	-	-	S	-	S	-	S	-	S	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

IV. Discussion:

In this study highest occurrence were observed in male as compare to female. It was correlated with siddqui etal (2002) study which shows male preponderance (72%). Maximum number of patient belongs to age group 0-10 years followed by 21-30 years. Findings were compared to other authors like V.G bhatt & S.D Vassikar (2010) and S. Rajeshwar Rao etal. (2014) showed similar results. Klebsiella spp. was the most predominant organism 28 (28%). My study is correlated with other workers like Dr. R. Sarathbabu etal. (2012), Rajeshwar Rao etal. (2014), Kritu Panta etal. (2013) and K.N.Ravichitra (2014) have found Klebsiella spp. as the predominant organism present in wound infection. Pseudomonas aeruginosa was the second most common organism 20 (20%) in the present study. This finding is correlated with other workers like A.Ananth and S. Rajan (2014), Sankarankutty J etal., (2014), Aizza Zafar (2008) also shown as second most disease causing organism. However many workers have found most predominant organism in their studies like Farzana R etal. (2013), Ehmer Al-Ibran (2013) and Soumya Kaup and Jaya Sankarankutty (2014). Staphylococcus aureus was the next common organism 8 (8%) in the present study and correlated with other workers like Haitham M.etal. (2011), Iman A. Hussain etal. (2012).

The present study reveals the incidence of infections due to Klebsiella and their tendency towards antibiotic resistance. Multidrug resistant bacteria are emerging worldwide which causes major public health problems and challenges to health care.

Klebsiella pneumoniae is 100 % sensitive to Imepenem, 71.42% of Amikacin and Cefotaxime, 67.5% Ceftazidime ,60.71% Ciprofloxacin, 53.57 %, Tetracycline and Amoxyclave. Pseudomonas aeruginosa is 100% sensitive to Imepenem, and Amikacin followed by Piperacilline (75%), Ceftazidime (65%), Amoxyclave, Ciprofloxacin and cefotaxime (60%) and Tetracycline (50%). Staphylococcus aureus 100 % sensitive to vancomycin, 87.5% to Linezolied, 62.5% to gentamicin, 62.5% to amoxyclave, 25% to oxacilline, 75% to ciprofloxacin 75% to erythromycin and 25%to Cotrimoxazole. These studies were compared to other studies like Soumya Kaup and Jaya Sankarankutty (2014), Rajeshwar etal.(2014) showed similarities in antibiotic sensitivity pattern.

V. Conclusion:

This study revealed the presence of wound infection causing bacteria, those are capable of causing various human illness. The commonest isolates of Wound infection are Klebsiella pneumoniae followed by Pseudomonas aeruginosa. Hence, Knowledge of the most common causative agents of infection and their antimicrobial susceptibility pattern is very essential for the judicious administration of empirical therapy before the culture results are available. Antimicrobial susceptibility of microorganisms varies from time to time and from place to place. Hence regular monitoring of bacterial susceptibility to antibiotics is essential. Antibiograms should be prepared regularly and made readily available to the clinicians to guide them in therapy. There is a need for a central database in India where various laboratories can upload their antibiogram regularly and this data can be very useful in formulating guidelines for treatment of various infectious diseases.

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