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ANTIBIOTIC SENSITIVITY TREATMENT FOR GRAM NEGATIVE BACTERIA ISOLATED FROM PUS SAMPLE

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ABSTRACT

A wide range of clinical samples pus were screened for identification of gram negative bacteria like Escherichia coli, Klebsiella sp. Pseudomonas aeruginosa and Proteus sp. their antibiotic sensitivity profile was evaluated. Out of 245 pus samples screened, one hundred and sixteen pure bacterial isolates was recorded. 64.65% of Enterobacteriaceae group was observed. It can be concluded that Gram negative bacteria were present in greater number than Gram positive bacteria in the pus sample. In these study Gatifloxacin(GT), Amikacin(Ak), Azithromycin(Az), Ampi/subbactum(As) and Ciprofloxacin(CF) showed the highest antibacterial activity to gram negative organism.

KEYWORDS

Staphylococcus aureu , Pseudomonas sp., E. coli, Klebsiella sp., Proteus sp.

1. INTRODUCTION

Pyogenic infection is characterized by several local inflammation, usually with pus formation, generally caused by one of the pyogenic bacteria, its can produce the accumulation of dead leukocytes and infectious agent commonly known as pus (Koneman, 2005). Its causes multiple abscesses or even empyema. Pyogenic infections are either polymicrobial or monomicrobial. An average of 5-6 strains of organisms is often involved in the infections with a mixture of aerobic and anaerobic organisms (Jeffrey *et. al.,* 1997). Most common organism are observed like *Staphylococcus aureus*, *Klebsiella* sp., *Pseudomonas* sp., *Escherichia coli*, *Proteus* sp., *Enterococci* sp., *Streptococci* sp. (Krige, J.E.J., 2001) and *Staphylococcus epidermidis* (Eugene J., *et. al*., 1997). For cure the problem antibiotic are main options. Selection of an effective antimicrobial agent for a microbial infection requires knowledge of the potential microbial pathogen, an understanding of the pathophysiology of the infectious process and an understanding of the pharmacology and pharmaco kinetics of the intended therapeutic agents (Kelwin, 1999). Hence the treatment of infection in patients becomes difficult. Studies are required to assess the right kind of antibiotics and the appropriate concentrations to be used in infections, taking into consideration the etiology of the infection and the duration of the antibiotic treatment. The study aimed to determine their antibiotic sensitivity and resistance pattern against used standard antibiotics. In this study bacterial pathogens showed resistance to most of the antibiotics.

2. MATERIAL AND METHOD

2.1 Sample collection, Isolation, Identification: The pus samples were collected from the outpatient departments of the Pt. Jawahar lal Nehru memorial medical college and Dr. B.R.A.M. hospital, Raipur (C.G.). India. The pus samples were examined for its odour, colour, presence of tissue and blood. The initial characterizations of the organisms present in the pus samples were carried out by direct microscopic examination using staining technique. Using selective plate technique did secondary analysis. The usages of selective media suppress the growth of the unwanted bacteria and perform biochemical tests for confirmation.

2.2 Preparation of Bacterial Strains Inoculums: The isolated bacterial strains; *Escherichia coli*, *Klebsiella* sp. *Pseudomonas aeruginosa and Proteus* sp. inoculums were prepared in 5 ml nutrient broth with 3 to 5 colonies of each bacterial strain. The inoculums were incubated at 37°C for 24 hr to get sample approximately close to 0.5% Mc Farland standard for susceptibility testing (NCCLS, 1993).

2.3 Antibiotic Discs Used: Commercially available antibiotic discs for gram negative bacteria such as amikacin(Ak) 30µg, ampi/subbactum(As) 10µg, azithromycin(Az) 15µg, ciprofloxacin(CF) 10µg, cefotoxime(Cf) 30µg, ceftazidime(Cz) 30µg, ceftriaxone(CT) 30µg, gentamycin(G) 10µg, ofloxacin(OF) 5µg, tobramycin(TB) 10µg, gatifloxacin(GT) 5µg, levofloxacin(Le) 5µg, moxifloxacin(Mf) 5µg, nitrofurantion(Nf) 300µg, nettilin(NT) 30µg, norfloxacin(NF) 10µg were used. A sterile cotton swab was dipped into the cell suspension of the respective isolate whose turbidity was checked with 0.5% McFarland's standard and inoculated on the entire agar surface of each plate first in a horizontal direction and then in a vertical direction to ensure even distribution of the organisms. Antibiotic discs are placed after 5 min. to allow the agar surface to dry. The inoculated plates were incubated at 37°C for 24-48 hr in an inverted position and the zone of inhibition was recorded. The zone of inhibition was expressed in terms of the Mean ± Standard Deviation by using four replicas and the results were tabulated.

3. RESULT AND DISCUSSION

Pus infection patients are subjected to several factors that may be associated with multidrug resistant microorganism carriage such as inappropriate antibiotic treatment, chronic course of the wound and frequent hospital admission (Kandemir *et al.,* 2007). There are 254 swab examined and swabs yields growth 116 (47.3%) isolates. This means that sample yielded more than one organism (Mordi and Momoh, 2009). 52.65% wound swabs failed to yield any growth. This could be due to normal healing process where the bacteria have been overpowered by body's defence mechanism, antimicrobial activity in patients circulation since all of them had been on antibiotic therapy post operatively at time of collecting the samples or adequate nursing care e.g. use of antiseptics for cleaning the wounds. It is also possible that some organisms could have been anaerobic bacteria that were missed as cultures were incubated aerobically. This condition could not therefore support growth of such organisms.Sharma *et al*., reported that the bacterial isolates; *Staphylococcus aureus*, *Pseudomonas* sp. and *Proteus* sp. are present in pus. *Pseudomonas* sp., *E. coli, Klebsiella* sp. and *Proteus* sp. showed the high degree of sensitivity to different durg show in **Table no. 2**. In these study Gatifloxacin(GT), Amikacin(Ak), Azithromycin(Az), Ampi/subbactum(As) and Ciprofloxacin(CF) showed the highest antibacterial activity to gram negative organism. The pre operative antibiotics that the patients received were gentamicin/crystalline penicillin, ampiclox, amoxycillin, ampicillin, metronidazole and others. The most probable reason for their choice being that these antibiotics have been on market for long, they are readily available and relatively cheap (WHO, 1991). It was reported that amikacin (Raja, 2007) are the most effective agents against whole Gram-negative organisms. According to Seppala *et al.,* 1992 majority of gram-negative bacteria isolated were sensitive to gentamicin, ceftazidime and ciprofloxacin. However, most of the gram-negative bacteria isolated were resistant to ampicillin, chloramphenicol and amoxycillin. Ceftazidine and Ciprofloxacin are third-generation cephalosporins that are relatively rare in the hospitals and are expensive. Their high cost and being less readily available to patients means these drugs have not been misused and hence are more effective compared to those that have been in use for quite a long time (JR Anguzu and D Olila, 2007).

Table no. 1: List of zone size of antibiotic and sensitive antibiotic

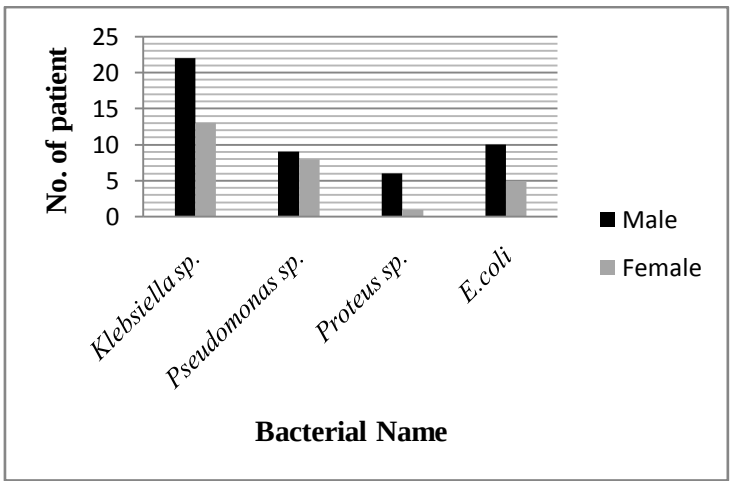
S.No.	Name	Standard			Sensitive Antibiotic		
		Zone in mm					
		R	IS	S	R	IS	S
1.	Amikacin(Ak)	14	15-16	17	39	5	**31**
2.	Azithromycin(Az)	13	14-17	18	42	17	**16**
3.	Ampi/subbactum(As)	11	12-14	15	52	9	**14**
4.	Ceftazidime(Cz)	14	15-17	18	**64**	3	8
5.	Ciprofloxacin(CF)	15	16-20	21	50	10	**15**
6.	Ceftriaxone(CT)	13	14-20	21	**73**	1	1
7.	Cefotoxime(Cf)	14	15-22	23	**74**	0	1
8.	Tobramycin(TB)	12	13-14	15	**72**	1	2
9.	Levofloxacin(Le)	13	14-16	17	**68**	3	4
10.	Ofloxacin(OF)	12	13-15	16	**74**	0	1
11.	Moxifloxacin(Mf)	14	15-17	18	**65**	6	4
12.	Nitrofurantion(Nf)	14	15-16	17	**62**	6	7
13.	Gentamycin(G)	12	13-14	15	67	0	8
14.	Gatifloxacin(GT)	12	13-14	15	39	0	1
15.	Nettilin(NT)	15	16-17	19	56	4	**15**
16.	Norfloxacin(NF)	12	13-16	17	**63**	4	8

R=Resistance, IS=Intermediated sensitive, S=Sensitive

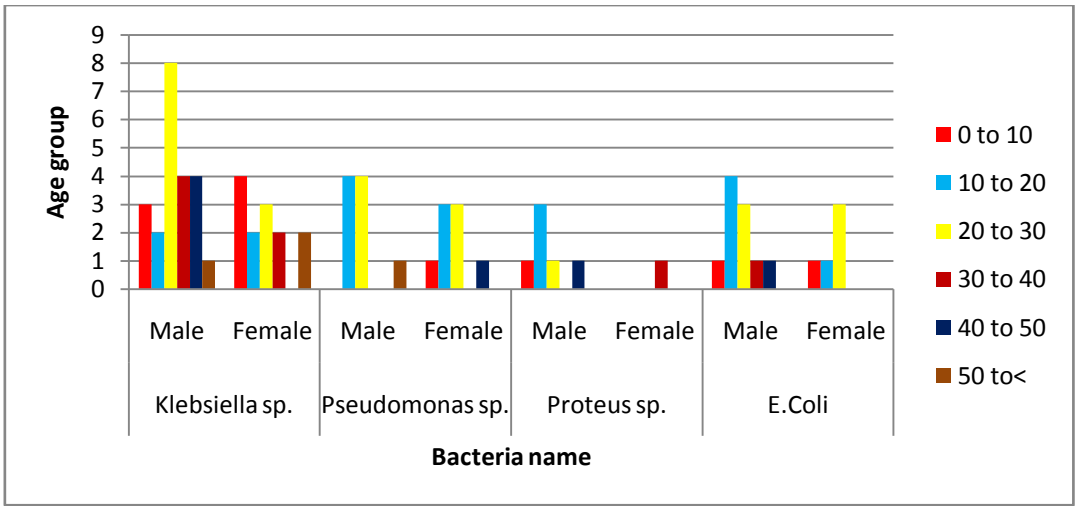
Table no. 2: List of sensitive antibiotic in gram negative bacteria

S.No.	Isolate	Sensitive Antibiotic															
		Ak	Az	As	Cz	CF	CT	Cf	TB	Le	OF	Mf	Nf	G	GT	NT	NF
1	*P.* sp.	2	2	1	0	2	0	0	0	0	0	0	0	0	3	1	0
2	*K.*sp.	17	7	8	3	6	6	1	2	2	1	5	1	4	24	6	9
3	*E. coli*	7	3	3	2	1	0	0	0	1	0	1	5	1	11	5	1
4	*Ps.*sp.	3	3	2	2	6	0	0	0	1	1	1	1	2	6	3	1

P= Proteus sp., *K.*sp.= *Klebsiella* sp., *Ps.*sp.= *Pseudomonas* sp.



Graph no 1: no. of isolates in male and female



Graph no 2: no. of patient in different isolates and different age group

4. CONCLUSION

In conclusion Proper management of pus infection with the appropriate antibiotic must be implanted keeping in mind the incidence of drug resistance in this population.

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