Original Research Article

A study to evaluate the shifting trend of antimicrobial resistance

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Abstract

Introduction: Pus is an important clinical specimen received in Microbiology laboratory for aerobic culture and sensitivity. The pace of development of drug resistance in bacterial isolates far exceeds the rate of newer drug discoveries highlighting the importance of conducting periodic studies to determine their antibiogram.**Aim:**This study was conducted to evaluate the shifting trend of antimicrobial resistance in various pus isolates.**Materials and Methods:** Pus samples from different departments of Patna Medical College and Hospital, Patnawere collected using aseptic technique for 6 months duration and were processed in the laboratory immediately using standard microbiological procedures. Identification of bacteria was carried out with motility testing, Gram staining and biochemical reactions. The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer's disc diffusion method on Muller Hinton agar and interpreted as per CLSI guidelines. **Results:** Analysis of 119 pus samples showed 93.27% culture positivity (M:F=1.48:1.00) with Surgical wards(32.43%) being the major contributor. Pseudomonas(29.73%) was the most common organism followed by Staphylococcus aureus(22.52%).Gram positive cocci were susceptible to Linezolid(94.87%), Vancomycin(92.31%) and Imipenem(92.31%) whereas Gram negative bacilli were susceptible to Imipenem(87.10%), Piperacillin(61.29%) and Gentamicin(48.39%). **Conclusion:**The changing trends of antimicrobial susceptibility in bacterial isolates from paus can serve as a useful tool for physicians to start empirical treatment of patients at the earliest according to the geographical areas and emerging multi- resistant bacteria.

Keywords: Antibiogram, Imipenem, Pseudomonas, Pus, Staphylococcus aureus.

Introduction

Pyogenic infections are characterized by local and systemic inflammation usually with pus formation [1]. These may be endogenous or exogenous. A break in the skin can provide entry to the surface bacteria which thereby start multiplying locally. The body's defense mechanism includes bringing immune cells into the area to fight against bacteria. Eventually, accumulation of these cells produces pus which is a thick whitish liquid[2].

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The inadvertent use of antibiotics leads to emergence of drug resistant pathogens, which in turn acts as a great challenge to the health services. Moreover, highly virulent strains and capacity to adapt quickly to changing environment worsens the situation and draws a matter of concern [3].Different studies have been conducted across the globe from time to time to assess the bacterial profile and the antibiotic susceptibility pattern in pus samples. This is particularly relevant for the treating physician who needs to start empirical treatment of patient until the lab culture reports are awaited[4].

Though the bacterial profile from pus samples remain similar in various studies, but there is a considerable variation in the antibiotic susceptibility pattern of theses isolates highlighting the increasing threat of

emergence of resistant bacteria and hence a need for a continuous surveillance of such changing trends. Therefore, this study was conducted to evaluate the shifting trend of antimicrobial resistance in various pus isolates.

Material ans methods

This is a prospective, unicentric study was conducted at Department of Microbiology, at Patna Medical College and Hospital, Patna. The study was conducted over a period of 06 months, from April , 2020 to October, 2020. The study was approved by institutional ethical and research committee. Informed and written consent was obtained from all the participating subjects, prior to commencement of the study.[5]A total of 119 pus samples obtained for aerobic culture and sensitivity from different IPDs & OPDs of our institution. Pus samples were collected with sterile disposable cotton swabs and aspirates in syringe and were transported and processed in the microbiology laboratory immediately. They were inoculated on to Blood agar (BA), Mac Conkey agar (MA) and Nutrient agar (NA). Culture plates were incubated at 37°C for 24 hrs to 48 hrs in aerobic condition. After incubation, identification of bacterium from positive cultures was done with a standard microbiological technique which includes motility testing by hanging drop preparation, gram staining and biochemical reactions such as catalase, coagulase, indole, methyl red, Voges-Proskauer, citrate, urease, phenyl pyruvic acid test and oxidase test [6].

The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer's disc diffusion method [7] on Muller Hinton agar and interpreted as per CLSIguidelines[8] and classified as sensitive, intermediate and resistant. Standard antibiotics amoxicillin (30mcg), amoxyclav (30mcg), ceftriaxone (30mcg), cefadroxil (30mcg),cefoperazone (75mcg), gentamicin (10mcg), imipenem (10mcg), methicillin (5mcg), linezolid (30mcg), ofloxacin (5mcg), vancomycin (10mcg), piperacillin (100mcg), ticarcillin (75mcg) and aztreonam (30mcg) were tested. S.aureus ATCC 25923 and E.coli ATCC 25922 were used as quality control [9]. All the culture media, biochemical media and antibiotics used were obtained from Hi Media.

Statistical analysis:Results obtained were analyzed by counts and percentages using MS Excel, 2010 version.

Results

Out of 119 pus samples obtained in the Microbiology lab from various departments of Patna Medical College and Hospital, Patnafor aerobic culture and sensitivity, 111 (93.27%) samples yielded a positive culture whereas 8 (6.73%) samples yielded no growth. Among 111 samples, 64 (57.66%) were male patients and 47 (42.34%) were female patients (Table 1) giving a male: female ratio of 1.48:1.00 and the isolate distribution is shown in figure 1.

Table 1: Sex-wise distribution of positive cultures obtained from pus samples

Sex	Culture positive (n=111)
MALE	64
FEMALE	47

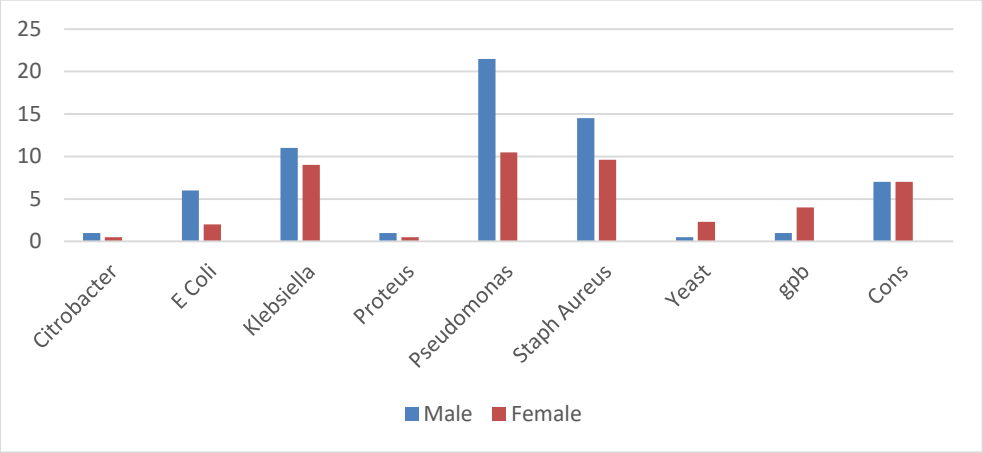

Fig. 1: Aerobic bacterial Isolates from Pus Culture

The department-wise distribution showed that surgery (32.43%) department was the major contributor of pus samples followed by ENT (30.63%), medicine (22.52%), orthopedics (11.71%), ICUs (6.31%) and others (2.70%). The most predominant gram positive bacteria isolated was Staphylococcus aureus (22.52%) and predominant gram negative bacteria was Pseudomonas (29.73%) apart from other isolates such as Citrobacter (0.90%), Escherichia coli (7.21%), Klebsiella spp (18.92%), Proteus (0.90%), coagulase negative staphylococcus (12.61%), gram positive bacilli (5.41%). Two fungal isolates identified as Candida spp (1.80%) were also isolated.The antibiogram of gram positive cocci (Table 2a, 2b) revealed that Linezolid (94.87%) was the most susceptible drug followed by Vancomycin (92.31%) and Imipenem (92.31%). Gram negative bacteria (Table 3) of Enterobacteriaceae were most susceptible to Imipenem (87.10%) followed by Piperacillin (61.29%) and Gentamicin (48.39%). Pseudomonas spp (Table 4) were also susceptible to Imipenem (93.94%), Piperacillin (60.61%) and Gentamicin (42.42%).

Table 2a: Antibiotic susceptibility pattern of Gram Positive Cocci (n=39)-*Staphylococcus aureus*

Bacteria	*Staphylococcus Aureus* (n=25)					
	Sensitive		Intermediate		Resistant	
Antibiotics	Number	Percentage	Number	Percentage	Number	Percentage
Amoxicillin	1	4.00	1	4.00	23	92.00
Amoxyclav	10	40.00	1	4.00	14	56.00
Ceftriaxone	13	52.00	5	20.00	7	28.00
Cefadroxil	12	48.00	3	12.00	10	40.00
Cefoperazone	10	40.00	5	20.00	10	40.00
Gentamicin	20	80.00	1	4.00	4	16.00
Imipenem	22	88.00	1	4.00	2	8.00
Methicillin	9	36.00	3	12.00	13	52.00
Linezolid	23	92.00	1	4.00	1	4.00
Ofloxacin	18	72.00	2	8.00	5	20.00
Vancomycin	22	88.00	1	4.00	2	8.00

Table 2b: Antibiotic susceptibility pattern of Gram Positive Cocci (n=14)-Coagulase negative staphylococcus

	Coagulase negative staphylococcus (n=14)					
	Sensitive		Intermediate		Resistant	
Antibiotics	Number	Percentage	Number	Percentage	Number	Percentage
Amoxicillin	10	71.43	2	14.29	2	14.29
Amoxyclav	12	85.71	2	14.29	0	0.00
Ceftriaxone	12	85.71	1	7.14	1	7.14
Cefadroxil	10	71.43	3	21.43	1	7.14
Cefoperazone	11	78.57	2	14.29	1	7.14
Gentamicin	14	100.00	0	0.00	0	0.00
Imipenem	14	100.00	0	0.00	0	0.00
Methicillin	11	78.57	2	14.29	1	7.14
Linezolid	14	100.00	0	0.00	0	0.00
Ofloxacin	13	92.86	0	0.00	1	7.14
Vancomycin	14	100.00	0	0.00	0	0.00

Table 3: Antibiotic susceptibility pattern of Gram Negative Bacteria (n=31) other than pseudomonas

	Antibiogram of Enterobacteriaceae (n=31)					
	Sensitive		Intermediate		Resistant	
Antibiotics	Number	Percentage	Number	Percentage	Number	Percentage
Aztreonam	7	22.58	2	6.45	22	70.97
Piperacillin	19	61.29	7	22.58	5	16.13
Imipenem	27	87.10	0	0.00	4	12.90
Gentamicin	15	48.39	5	16.13	11	35.48
Ceftriaxone	13	41.94	6	19.35	12	38.71
Cefadroxil	8	25.81	2	6.45	21	67.74
Cefoperazone	16	51.61	4	12.90	11	35.48
Ofloxacin	14	45.16	5	16.13	12	38.71

Table 4: Antibiotic susceptibility pattern of Pseudomonas (n=33)

	Antibiogram of Pseudomonas(33)					
	Sensitive		Intermediate		Resistant	
Antibiotics	Number	Percentage	Number	Percentage	Number	Percentage
Piperacillin	20	60.61	4	12.12	9	27.27
Ticarcillin	10	30.30	1	3.03	22	66.67
Imipenem	31	93.94	0	0.00	2	6.06
Gentamicin	14	42.42	7	21.21	12	36.36
Ceftriaxone	7	21.21	2	6.06	24	72.73
Cefadroxil	5	15.15	3	9.09	25	75.76
Cefoperazone	10	30.30	7	21.21	16	48.48
Ofloxacin	8	24.24	1	3.03	24	72.73

Discussion

Gram negative bacteria such as Pseudomonas, Escherichia coli, Klebsiella spp and gram positive cocci such as Staphylococcus aureus are the common causative agents of various pyogenic infections. The emerging resistant genes in such bacteria by various mechanisms are a matter of concern. In our study, a dominance of gram negative bacteria as the causative agent of pyogenic lesions is seen which is supported by Zubair et al [10]. Staphylococcus aureus (22.52%) is the most common gram positive isolate in our study as shown in studies of Tiwari et al [11] and Lee C Y et al [12] also and prevalence of MRSA is 35.90% similar to Pramila et al. Pseudomonas (29.73%) is the most common gram negative bacterial isolate which is in accordance with the report of Basu et al[13].The present study revealed that the male: female distribution of pus isolates to be 1.48:1 which closely corroborates with the study by Pappu A.K. et al [14]. Surgical ward had given maximum number of pus samples followed by ENT department.Staphylococcus aureus was susceptible to linezolid (94.87%) and Vancomycin (92.31%) contrary to 100% sensitivity in study of Samra et al [15]. Antibiotic sensitivity profile of gram negative bacteria showed sensitivity towards imipenem (87.10%), piperacillin (61.29%) and gentamicin (48.39%) as salso seen by Balan et al [16].The emergence and proliferation of these highly resistant organisms obtained from pus samples is highly threatening given the limited number of antimicrobial agents that are currently available or in the drug development pipelines of the pharmaceutical industry to combat these organisms.Every effort needs to be made to carefully select antibiotics, balancing the need for a broad spectrum of empiric coverage of potential microorganisms with the need to preserve available antibiotics for when they are absolutely necessary [17]

Conclusion

This study shows that pyogenic infections are an important cause of morbidity in patients with Gram negative bacteria (Pseudomonas) more predominant as compared to gram positive organisms (Staphlococcus aureus).A changing trend in antibiotic sensitivity profile of the isolates need to be monitored as there is limited availability of newer drugs and the emergence of resistant bacteria far exceeds the rate of new drugdevelopment.

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