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Prevalence of Antimicrobial Resistance in Bacterial Isolates Causing Pyogenic Infection in Patients Attending A Tertiary Hospital of Northern india

KEYWORDS: Pyogenic Infection, Antimicrobial Susceptibility, Clinical Isolates.

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ABSTRACT ***Background:*** *Antibiotic resistance is a global concern and particularly pressing in developing nations, including India, where the burden of infectious disease is high and healthcare spending is low* ***Aim****: To find out the antimicrobial resistance in commonly encountered pathogens in pus samples.* ***Materials and methods****: This study was conducted from January 2015 to June 2015 in IIMS&R central lab. Total 128 pus samples were collected. Antimicrobialsusceptibility testing was done by Kirby-Bauer disk-diffusion method followed by culture and biochemical test.****Result****: A total of 96 samples were found positive in which 56 (58.3%) were male and 40(41.6%) were females yielding a male: female ratio of 1.4.The most common organism isolated was found to be S. aureus 42 (38.18%),followed by Klebsiella spp 14 (12.72%), Pseudomonas spp13(11.83%) & Escherichia coli 11(10%).Gram positive isolates showed 100 % sensitivity with Vancomycin, Linezolid and Teicoplanin.* ***Conclusion****: The knowledge of prevalent local pathogens and their antibiogram will help the clinician to choose the appropriate antimicrobial agent for effective and rationale treatment.*

INTRODUCTION

Pyogenic infection is characterized by severe local inflammation, usually with pus formation, and generally caused by pyogenic bacteria[1]. Pus is a collection of thick, white or yellow fluid that accumulates around the source of infection. Pus is made up of dead tissue, white blood cells, and damaged cells. Breaking of the protective layer during trauma, accident, minor injury may induce variety of cell types by the host response leading to pus formation[2]**.** Pyogenic infection is one of the most frequent types of nosocomial infection in developing countries[3].Most commonly encountered pathogen in pus is *Staphylococcus aureus* followed by member of family *Enterobactericeae eg. Escherichia coli, Proteus* etc. *Staphylococcus aureus* is one of the most versatile nosocomial pathogen and is responsible for more than 80 percent of the suppurative diseases encountered in medical practice[4].

Large numbers of *Staphylococci* are disseminated in pus and exudate discharged from large infected wounds, burns and secondarily infected skin lesions[5].Multidrug resistant organisms including *Staphylococci* are mainly acquired in hospital settings; where these organisms are treated by prolong use of broad spectrum antimicrobials. Healthcare workers are also the source of transportation of bacteria by picking them from one patient and passing it on to other patient[6].Pyogenic infections have been a problem in the field of medicine for a long time. Advances in control of infections have not completely eradicated this problem because of development of drug resistance[7].

Knowledge of the causative agents of wound infection has proven to be helpful in the selection of appropriate antimicrobial therapy and on infection control measures taken in health institutions [8]. This study has been designed to evaluate the profile of isolates causing pyogenic infection and their resistance to various antimicrobial agents.

Material and Methods:

The present study is cross-sectional study of clinically suspected cases of pyogenic infection attending Integral Institute of Medical Sciences Research. Study period was 6 months from January 2015 – June 2015**.** Total 128 pus samples were included in this study. These samples were obtained from drained Abscess 35 (27.34%), Cellulitis 26 (20.31%), Traumatic wound 25 (19.53%), Surgical site infection 15 (11.72%), CSOM 10 (7.81%), Osteomyelitis 6 (4.69%), Diabetic foot 6 (4.69%) and ASOM 5 (3.90%)**.** For laboratory investigation, two pus swabs were collected; one for the direct smear and the other for culture. The pus specimens were cultured onto the MacConkey agar and Blood agar plates and incubatedat 37°C for 24 to 48 hours. After overnight incubation, the culture plates were examined for bacterial growth and identified using standardmicrobiological techniques.

Antimicrobial susceptibility testing (AST): Antimicrobial susceptibility testing was performed by Kirby bauer disk diffusion and results were interpreted as per CLSI guidelines. Antibiotic discs were procured from HiMedia Mumbai India.

Statistical analysis: Data was analyzed by using SPSS of version 21.0 (IBM). MS. Excel was used for graphical presentation. Results are presented in proportion or percentage form.

Results: This study is an attempt to estimate the prevalence of antibiotic resistance in patients attending IIMSR, Hospital. Total 128 pus samples were included in this study. Out of 128 samples 96 samples were culture positive. Department wise distribution of pus samples revealed that highest contribution was from Surgery 42 (35.15%) followed by Orthopedics 31 (24.21%), ENT 20 (15.62%), Medicine 16 (12.5%), Obs&Gynae12 (9.37%). Least samples were collected from Pediatrics4 (3.12%) department**(Table–1)**. Among total 128 pus sample, 73 were obtained from male patients and 55 were obtained from female patients. Out of 73 male patients 56(76.71%) were culture positive and among 55 female patients 40(72.72%) were culture positive, yielding a male: female ratio of **1.4. (Table–2).**Out of 110 bacterial isolates *Staph*

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aureus was most common isolate 42 (38.18%) followed by *Klebsiella spp*14 (12.72%), *Pseudomonasspp*13(11.83%), *E. coli* 11(10%), *Enterococcus spp* 7(6.36%), *Proteus spp* 5(4.55%), *Citrobacterspp5 (4.55%),,Streptococcus pyogenes*4(3.63%), *Acinetobacterspp*3(2.72%), *Streptococcus pneumoniae*2(1.82%),*CoNS*2(1.82%) and *Diphtheroides*2 (1.82%). The antimicrobial sensitivity pattern of Gram Positive bacteria showed (100%) sensitivity against Vancomycin, Linezolid and Tecoplanin **(Table-3 and Table-4)**. Antimicrobial sensitivity pattern of E.coli showed 100% sensitivity against Imipenem followed by Amikacin (82%) and Piperacillin -tazobactum (82%)**(Table-5)**.*Pseudomonas spp* were 100% susceptible to Polymyxin B and 92% withImipenem/ cilastatin (**Table-6)**

Table-1: Distribution of Samples according to Department

Department	n (%)
Surgery	45 (35.15)
Orthopaedics	31 (24.21)
ENT	20 (15.62)
Medicine	16 (12.5)
Obstetrics &Gynaecology	12 (9.37)
Paediatrics	4 (3.12)
Total	128 (100)

Table-2: Pure and Mixed Growth of Bacterial Isolates

S.no.	Bacterial Isolates	Pure growth n (%)	Mixed growth n (%)	
1	S. aureus	36(43.90)	E.coli+Klebsiella	2(14.26)
2	Pseudomonas	11(13.41)	S.aureus + Klebsiella	2(14.26)
3	E.coli	8(9.76)	Klebsiella+Citrobacter	2(14.26)
4	Klebsiella	7(8.54)	S.aureus+ S.pyogens	2(14.26)
5	Enterococcus	4(4.88)	Enterococcus+ Proteus	1(7.14)
6	Proteus	4(4.88)	S.pneumoniae+ S.aureus	1(7.14)
7	Acinetobacter	3(3.66)	S. aureus+Pseudomonas	1(7.14)
8	Citrobacter	3(3.66)	S.pyogenes+Enterococcus	1(7.14)
9	CoNS	2(2.44)	E. coli+Enterococcus	1(7.14)
10	Diptheroids	2(2.44)	Pseudomonas+Klebsiella	1(7.14)
11	S. pyogenes	1(1.22)		
12	S. pneumoniae	1(1.22)		
Total		82(100)		14(100)

Table 3: Antibiotic susceptibility pattern of Gram- positive bacteria (except *Enterococcus spp*)

Name of antibiotics	S. aureus (42)		CoNS (2)		S. pyogenes (4)		S.pneumonia(2)	
	S n(%)	R n(%)	S n(%)	R n(%)	S n(%)	R n(%)	Sn(%)	R n(%)
Penicillin	2 (5)	40 (95)	0	2 (100)	4 (100)	0	2 (100)	0
Oxacillin	30 (71)	12(29)	2(100)	0	0	0	2 (100)	0
Amoxicillin-clavulanic acid	18(43)	24(57)	1(50)	1(50)	4 (100)	0	2 (100)	0
Trimethoprim/ Sulfamethoxazole	13 (31)	29(69)	2 (100)	0	3(75)	1 (25)	2 (100)	0
Erythromycin	29(69)	13 (31)	2 (100)	0	3 (75)	1 (25)	2 (100)	0
Gentamycin	33 (79)	9 (21)	2 (100)	0	4 (100)	0	2 (100)	0
Amikacin	37 (88)	5 (12)	1 (50)	1 (50)	4 (100)	0	2 (100)	0
Ciprofloxacin	10 (24)	32 (76)	0	2 (100)	3 (75)	1(25)	2 (100)	0
Clindamycin	37 (88)	5(12)	2 (100)	0	4 (100)	0	2 (100)	0
Vancomycin	42 (100)	0	2 (100)	0	4 (100)	0	2 (100)	0
Linezolid	42 (100)	0	2 (100)	0	4 (100)	0	2 (100)	0
Teicoplanin	42 (100)	0	2 (100)	0	4 (100)	0	2 (100)	0

(S= sensitive , R= resistance)

Table- 4: Antibiogram of *Enterococcus*spp

Name of antibiotics	Enterococcus spp(7)	
	Sensitive n(%)	Resistance n(%)
Penicillin	5 (71)	2 (29)
Ampicillin	7 (100)	0
Vancomycin	7 (100)	0
Teicoplanin	7 (100)	0
Linezolid	7 (100)	0
High level Gentamycin	4 (57)	3 (43)
High level Streptomycin	4 (57)	3 (43)
Ciprofloxacin	4 (57)	3 (43)
Levofloxacin (LE)	4 (57)	3 (43)
Tetracycline (TE)	3 (43)	4 (57)
Doxycycline (DO)	4 (57)	3 (43)

Table 5: Antibiogram of Gram negative Bacteria (except *Pseudomonas*)

Name of antibiotics	E.coli (11)		Klebsiellaspp (14)		Citrobacterspp (5)		Proteus spp (5)		Acinetobacterspp (3)	
	S n(%)	Rn(%)	S n(%)	Rn(%)	S n(%)	Rn(%)	S n(%)	Rn(%)	S n(%)	R n(%)
Ampicillin	0	11(100)		14(100)	1(20)	4(80)	1(20)	4(80)	0	3 (100)
Ampicillin -sulbactum	2(18)	9(82)	4 (29)	10 (71)	1 (20)	4(80)	3 (60)	2 (40)	1 (33)	2 (67)
Amoxicillin -clavulanic acid	2(18)	9(82)	1(7)	13(93)	1(20)	4(80)	1 (20)	4 (80)	1 (33)	2 (67)

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Piperacillin -tazobactum	9(82)	2(18)	4 (29)	10 (71)	2 (40)	3(60)	4 (80)	1 (20)	1 (33)	2 (67)
Ticarcillin - clavulanic acid	6 (54)	5(46)	3 (21)	11 (79)	1(20)	4(80)	3 (60)	2(40)	0	3 (100)
Cefotaxime	1 (9)	10 (91)	2 (14)	12 (86)	1 (20)	4 (80)	2(40)	3 (60)	0	3 (100)
Ceftazidime	2 (18)	9 (82)	2 (14)	12 (86)	1(20)	4 (80)	2 (40)	3 (60)	0	3 (100)
Cefixime	0	11(100)	2 (14)	12 (86)	1 (20)	4 (80)	2 (40)	3 (60)	0	3 (100)
Cefepime	2 (18)	9 (82)	1 (7)	13 (93)	1(20)	4 (80)	1 (20)	4 (80)	0	3 (100)
Ceftazidime/ clavulanic acid	8 (73)	3 (27)	4 (29)	10 (71)	2 (40)	3(60)	4 (80)	1 (20)	2 (67)	1 (33)
Ceftriaxone -sulbactum	8 (73)	3 (27)	5 (36)	9 (64)	1 (20)	4 (80)	4 (80)	1 (20)	1 (33)	2 (67)
Cefotaxime - clavulanic acid	9 (82)	2 (18)	6 (43)	8 (57)	1 (20)	4 (80)	4 (80)	1 (20)	2 (67)	1 (33)
Chloremphinocal	9 (82)	2 (18)	5 (36)	9 (64)	1 (20)	4 (80)	2 (40)	3 (60)	1 (33)	2 (67)
Trimethoprim/ sulfamethoxazole	1(9)	10 (91)	2 (14)	12 (86)	0	5 (100)	2 (40)	3 (60)	0	3 (100)
Tetracycline	2 (18)	9 (82)	8 (57)	6 (43)	3 (60)	2 (40)	2 (40)	3 (60)	1 (33)	2 (67)
Amikacin	9 (82)	2 (18)	6 (43)	8 (57)	2 (40)	3 (60)	3 (60)	2 (40)	1 (33)	2 (67)
Gentamycin	7 (64)	4 (36)	5 (36)	9 (64)	2 (40)	3 (60)	4 (80)	1 (20)	1 (33)	2 (67)
Tobramycin	8 (73)	3 (27)	6 (43)	8 (57)	2 (40)	3 (60)	2 (40)	3 (60)	2 (66)	1 (33)
Imipenem-cilastatin	11(100)	0	10 (71)	4 (29)	3 (60)	2 (40)	5 (100)	0	2 (67)	1 (33)
Meropenem	7 (64)	4 (36)	8 (57)	6 (43)	1 (20)	4 (80)	5 (100)	0	0	3 (100)
Ertapenem	8 (73)	3 (27)	8 (57)	6 (43)	2 (40)	3 (60)	5 (100)	0	1 (33)	2 (66)

Table 6: Antibiogram of *Pseudomonas*spp

Name of antibiotics	Pseudomonas spp (13)	
	Sn (%)	Rn (%)
Piperacillin	6 (46)	7(54)
Ticarcillin	5(38.5)	8(61.5)
Piperacillin-tazobactum	9 (69.2)	4 (30.8)
Ticarcillin- clavulanic acid	7 (53.8)	6 (46.2)
Ceftazidime	6 (46.2)	7(53.8)
Aztreonam	7 (53.8)	6 (46.2)
Meropenem	8 (61.5)	5(38.5)
Imipenem/cilastatin	12 (92.3)	1(7.69)
Tobramycin	8 (61.5)	5(38.5)
Amikacin	6 (46.2)	7(53.8)
Gentamycin	6 (46.2)	7(53.8)
Ciprofloxacin	2 (15.3)	11(84.6)
Polymyxin B	13 (100)	0

DISCUSSION: Pyogenic infection has been a major concern among health care practitioners not only in terms of increased trauma to the patient but also in view of its burden on financial resources and the increasing requirement for cost effective management within the health care system. This study is an attempt to evaluate common pathogenic bacterial isolates responsible for pyogenic infection and their resistance pattern.

The Department wise distribution of pus samples revealed that Surgery department was the highest contribution 45 [35.15%], followed by 31 (24.21%) Orthopedicsdepartment, 20 (15.62%) samples were from ENT department, 16 (12.5%) from Medicine, 12 (9.37%) fromObs&Gynae ward and 4 (3.12%) from Paediatric wards. This finding is in agreement with the study done by **Raghavet *al*[9]** which showed surgery department as the highest contributors [35.29%], followed by Orthopaedics [29.42%], Gynae& Obs. [11.76%], Medicine [9.80%], Skin [7.85%] and ENT [5.88%)]departments.

The present study revealed that the male is to female distribution of pus samples to be 1.4 which closely corroborates with the study done by **Pappuet *al*[10]** Among the 96 culture positive pus samples, 82 yielded pure bacterial growth and 14 yielded mixed growth. Over all total 110 organisms were isolated from 96 pus samples. Another study conducted by **Vermaet *al*[11]**reported that out of 245 pus specimen a total of 116 bacterial isolates were obtained among which 86 were monomicrobialand 16 were polymicrobial but no growth seen in 149 cases.

According to our findings pyogenic infection was more common in male than female the predominance of males cases is probably due to more exposure to the environment and more chances of accidents while earning livelihood.

In present study frequency of Gram positive organisms was found to be [53.63%] whereas the frequency of Gram negative organisms was [46.36%]. A study done by **Asatiet al[12]**in a tertiary Care Hospital, reported the frequency of gram positive organisms was found to be [45.2 %] whereas the frequency of gram negative organisms was [54.8%]. Gram positive dominance is seen in our Hospital because our center is secondary health center where community acquired infection is common.

S. aureus 42 [38.18%] was the most common pathogen cultured followed by *Klebsiellaspp*14 [12.72%], *Pseudomonas spp*13[11.83%] &*Escherichia coli* 11 (10%) which is in agreement with the study conducted by **Gupta *et al*[13]** He reported that the most common isolate from wound infection was *Staphylococcus aureus* [32.3%] followed by *Klebsiellapneumoniae*[22%], *Pseudomonas aeruginosa* [18.7%] &*Escherichia coli* [17.4%]. According to Centers for Disease Control and Prevention Atlanta Georgia, *Staphylococcus aureus* is the most prevalent organism associated with surgical wound infections.Gram positive bacteria showed 100% sensitivity to Vancomycin, Linezolid and Teicoplanin. These results were comparable to studies carried out by others **Anupurbaet *al*[14]** and **Priyaet *al*[15]**

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Priyaet *al* reportedthat Gram positive isolates were 100% sensitive to Vancomycin, Linezolid and Dalfopristin/Quinpristin.

Among 51 Gram negative isolates, majority of isolates [68.62%] belonged to *enterobacteriaceae*. Another study done by **Binduet *al*[16]**reported the prevalence of *enterobacteriaceae* in pyogenic infection to be (81.70%). In present study the majority of gram negative isolates were most sensitive to Imipenem-cilistatin [84.31%], followed by Amikacin [52.94%]. This is in agreement with the study done by **Raghavet *al*[9]** that gram negative isolates were most susceptible to Imipenem [80%], Amikacin [70%] and Piperacillin/tazobactam [70%]. Gram negative isolates in present study showed better sensitivity to Amikacin than Gentamycin.

Conclusion: This study reveals that a variety of bacterial pathogens are responsible for pyogenic infection in our center. Even though gram negative bacteria are being increased significantly but still *Staphylococcus aureus* is being continued as a major etiological agent of pyogenic infections.Emerging multidrug resistant strains is of major concern to treat these conditions. High level of resistance was seen to commonly used antimicrobial agent Penicillin, Amoxicillin, Ciprofloxacin and Ofloxacin. Our observations emphasize the need of continuous surveillance to monitor etiology and antimicrobial susceptibility patterns both in the community and hospital settings to guide the empirical use of antimicrobials. The study will guide the clinician in choosing appropriate antibiotics according to sensitivity pattern which will contribute to better treatment and judicious use will also help in preventing emergence of resistance to the drug which is still sensitive

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