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**Original Research Paper** 

Microbiology

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**ABSTRACT** Background and aim: Profile of microbiological agents and their susceptibility to different antibiotics have to be understood before staring proper treatment. The study was aimed to obtain microbiological profile of pus isolates, and to ascertain antibiotic susceptibility pattern of pus isolates.

**Methods:** A retrospective study was conducted in a tertiary care hospital, Dr. RPGMC Tanda. A total of 570 pus culture samples collected by sterile swab and sterile syringe aspiration from clinically suspected cases of pyogenic infections, from different wards, were processed by standard microbiological methods. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion test as per CLSI guidelines and were classified as sensitive or resistant.

**Results:** Out of 570 samples received in the department of Microbiology, Dr. RPGMC, Tanda for antibiotic culture and sensitivity, 288 (50.5%) samples yielded positive culture whereas 282 (49.5%) samples yielded no growth. Among 570 samples, 554 (97.1%) were adults whereas 16 (3.9%) were children. Out of 570 samples, 393 were males while 177 were females. Samples were mainly from orthopedics ward (52.6%) and surgery ward (21%) followed by medicine, obstetrics and dermatology wards. Growth was mono-microbial in 204 (70.8%) isolates, bi-microbial in 72 (25%) while poly-microbial in 12 (4.2%) pus isolates. Staphylococcus aureus was the most common gram-positive organism. Prevalence of MRSA was 66.84% among Staphylococcus. Among gram negative organisms, E. coli was most common followed by Pseudomonas species. Candida was the only fungal growth seen in 10 (2.6%) samples. Antibiotic susceptibility pattern of Staphylococcus showed 100% susceptibility to vancomycin. Among the AST profile of gram-negative organisms, E. coli showed 76.1% sensitivity to aminoglycosides while Pseudomonas showed 88.5% sensitivity to piperacillin.

**Conclusion:** A continuous inspection should be carried out to monitor the antimicrobial susceptibility of bacterial isolates from pus samples to choose appropriate antibiotics for prophylaxis and treatment of infections.

# **KEYWORDS** : Antibiotics, microbiology, resistance

# INTRODUCTION

Pyogenic infections refer to infection that causes pus formation and are characterized by local inflammation, usually by multiplication of microorganism.<sup>1</sup> Pus is a collection of thick, white or yellow fluid, formed at the site of inflammation during infection. It is made up of dead tissue, white blood cells, and damaged cells.<sup>2</sup> The occurrence of wound infections depends on various factors like condition of wound, microbial load and the host defense mechanisms.<sup>3</sup> The overall incidence of wound sepsis in India is from 10% to 33%.<sup>4</sup> The infecting pathogens not only differ from country to country, but also vary from one hospital to another within the same country.<sup>5</sup> It is caused by bacteria, virus, fungi and protozoa and in many cases there is a mixed infection with more than one bacterial species.<sup>6</sup>

The most common causative agent includes *Staphylococcus* aureus which account for 20-40% isolates. Infection with Pseudomonas aeruginosa occurs mainly following surgery and burns which account for 5-15%. Escherichia coli, Klebsiella sp., Proteus sp. and Enterococci sp. are commonly associated with pyogenic infections.<sup>78</sup>

Selection of an effective antimicrobial agent for a microbial infection depends on the causative agent, pathophysiology of the infectious process and on pharmacodynamics and pharmacokinetics of the antimicrobial agents. Also, antibiotic resistance to the commonly used antibiotics is now emerging as a result of misuse and abuse of particular antibiotics. <sup>9</sup> The routine use of antibiotics has resulted in wide spread antibiotic resistance especially within the gram-negative organisms.<sup>10</sup>

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. 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.

Bacteria have the ability to acquire resistance and can transfer the resistance from one bacterium to another.<sup>11</sup> Earlier, such multidrug resistant organisms were common in immunosuppressed patients but now, reports are showing such infections in normal healthy individuals as well. Also, such drug-resistant infections may complicate the newly emerging infectious diseases.<sup>12</sup> The emergence of high antimicrobial resistance among bacterial pathogens has made the management and treatment difficult.<sup>13</sup> It is ideal to give proper antibiotic after culture and sensitivity of the wound swab or pus.<sup>14</sup> The present study aimed to detect common bacteriological profile and their antibiotic susceptibility profile from wound infection at Dr RPGMC Kangra at Tanda.

# METHODS

A retrospective study was conducted at Department of Microbiology, Dr. RPGMC Kangra at Tanda. All pus samples collected during 3-months were included. Socio-demographic and laboratory results were collected from Hospital Microbiology Laboratory registration books by using a standard data collection format.

Pus samples were aseptically collected using sterile cotton swab and aspirates in syringe and were transported and processed in the microbiology laboratory immediately. They were inoculated on to Blood agar, MacConkey agar and Nutrient agar. Culture plates were incubated at 37°C for 24 hours to 48 hours 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.

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 (Clinical and Laboratory Standard Institute, 2017) and classified as sensitive and resistant. Detection of MRSA isolates were done by using Cefoxitin disc ( $30\mu$ g). S. aureus ATCC 25923 and E. coli ATCC 25922 were used as quality control.

Data were presented as frequency and percentages.

# RESULTS

Among 570 samples, 554 (97.1%) were adults whereas 16 (3.9%) were children. Out of 570 samples, 393 were males while 177 were females. Samples were mainly from orthopedics ward (52.6%) and surgery ward (21%) followed by medicine, obstetrics and dermatology wards.

Of 570 samples collected, 288 samples were culture positive. Out of 288 positive samples, mono-microbial, bi-microbial, and poly-microbial were 204, 72, and 12 respectively (Figure 1).

Among isolates, *Staphylococcus aureus* was the most common in 187 samples (MRSA 125 and MSSA 62) followed by E. coli in 45 samples, and non-fermenter bacteria in 37 samples (Table 1).

Antibiotic susceptibility pattern for Gram negative bacteria has been shown in table 2. Among 45 E. coli positive samples, 31 samples were sensitive for amikacin. Among 18 klebsiella positive samples, none of the sample was sensitive for cefixime. Out of 28 *Psuedomonas aeruginosa* positive samples, none of the samples was resistant for cotrimoxazole and cefixime.

Among all S. aureus samples, 169 were sensitive to Amikacin followed by cefoxitin (n=125). (Table 3) Among 125 MRSA, all isolated were sensitive to vancomycin.

# DISCUSSION

Pyogenic infections are characterized by local and systemic inflammation usually with pus formation. It may be either monomicrobial or polymicrobial. Gram negative bacteria such as Pseudomonas, Escherichia coli, Klebsiella spp., Proteus spp., and Gram-positive cocci such as Staphylococcus aureus and Enterococci are the common causative agents.<sup>14</sup>

In this study, both gram-positive and gram-negative pathogens were isolated from samples. The most common gram-negative pathogen was *E. coli* (15.6%) followed by nonfermenter (other than pseudomonas) (12.8%), and Pseudomonas species (9.7%). These organisms are commonly found in hospital environment. They tend to be resistant to common antimicrobial agents and are also multidrug resistant. Among gram-positive pathogens, Staphylococcus aueus was commonly isolated followed by Coagulase Negative Staphylococcus species and Enterococcus species which correlates with the study done by Kumari et al.<sup>15</sup> *S. aureus* being normal flora of the skin, is usually associated with pyogenic infections.<sup>16</sup>

MRSA strains are resistant to a large group of antibiotics called beta-lactams, including penicillin and cephalosporins. Methicillin resistance is caused by the acquisition of a mecA gene. This produces an alternative penicillin binding protein 2a (PBP2a), which has lower affinity for -lactam antibiotics. In present study, MRSA was detected in 67% of S. aureus isolates. All MRSA were sensitive to vancomycin. Most of Gram positive isolates were sensitive to amikacin (90%) and cefoxitin (66.8%) which is same as the results of studies conducted by Verma and Shittu AO et al.<sup>17,18</sup> As antibiotic resistance among microorganisms is increasing, it has become mandatory to select antibiotics properly and to administer it at appropriate dosage and duration. Our study also showed existence of high drug resistance to multiple antibiotics in E. coli, S. aureus, K. pneumoniae, and P. aeruginosa isolates from pus samples. Hence, formulation of antibiotic policies and infection control measures at institutional or higher level has to be considered essential.<sup>1</sup>

# CONCLUSION

Administration of right antibiotics for right patients with right dose, route and frequency is important to save the patient from harm. Changing antibiotic sensitivity patterns points towards reconsidering empirical antibiotic regimens and formulation of institutional antibiotic policy.

# Table 1: Profile of isolates

	n	%
S. aureus	187	64.93
Non-fermenter	37	12.85
Pseudomonas	28	9.72
E. coli	45	15.63
Klebsiella	18	6.25
Enterobacteria	4	1.39
Citrobacteria	23	7.99
Proteus	15	5.21
Enterococcus	6	2.08
Streptococcus	6	2.08
Candida	10	3.47
CONS	4	1.39
Diphtheroid	1	0.35

# Table 2: Antibiotic susceptibility pattern of Gram-negative isolates

	E. coli (n=45)	Klebsiella species (n=18)	Proteus (n=15)	Citrobacter (n=23)	Enterobacter (n=4)	P. aeruginosa (n=28)	Non- fermenter (n=37)
Ceftazidime	16	5	9	9	3	22	9
Cotrimoxazole	12	8	5	10	1	0	8
Cefixime	10	0	7	-	1	0	7
Amikacin	31	6	10	18	3	18	6
Ciprofloxacin	14	8	9	9	2	18	14
Doxycycline	10	5	15	15	2	3	3

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	n	%
Penicillin	11	5.88
Cotrimoxazole	65	34.76
Amikacin	169	90.37
Clindamycin	112	59.89
Erythromycin	97	51.87
Cefoxitin	125	66.84
300		

# Table 3: Antibiotic susceptibility pattern of S. aureus isolates (n=187)



# Figure 1: Distribution of samples on the basis of absence or presence of bacterium

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