

Identification and Antibigram of Gram Positive Bacterial Isolates from Pyogenic Samples in a Tertiary Care Hospital in Ajmer, Rajasthan

¹Dr.Sushila Saini, Resident Doctor, J.L.N. Medical College, Ajmer

²Dr. Jitendra Kumar Saini, Senior Resident, J.L.N. Medical College, Ajmer

³Dr. Ranweer, Assistant Professor, J.L.N. Medical College, Ajmer

Corresponding Author: Dr. Jitendra Kumar Saini, Senior Resident, J.L.N. Medical College, Ajmer

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Abstract

Introduction: Pus is an important clinical specimen received in Microbiology laboratory for culture and sensitivity. In pyogenic infections gram positive bacteria are an important cause of sepsis. These infections are difficult to treat because of increasing antibiotic resistance in the pathogens.

AIM: To screen various gram positive bacteria in the pus samples and determine their antibiotic sensitivity and resistance pattern against standard antibiotics.

Materials and Methods: It was a observational prospective study. A total of 352 pus samples received from various wards of JLN medical college & associated group of hospitals, Ajmer from Dec.2018 to June 2019 and were processed in the laboratory using standard microbiological procedures. Identification of isolates by using gram staining and biochemical tests .The antibiotic sensitivity testing of isolates were performed by Kirby- Bauer disc diffusion method on Muller Hinton agar and interpreted as per CLSI guidelines.

Result: Out of 352 samples aerobic bacterial isolates were present in 290(82.39%) with Surgical wards (63.06%) being the major contributor. *K. pneumoniae* (18.65%) was the most common organism followed by *P. aeruginosa* (18.30%), *S. aureus* (16.28%), CoNS 42 (14.24%), *E. coli* 39(13.22%), *E. aerogenes* 29(9.83%), *Enterococci* 18(6.10%), *P. mirabilis* 6(2.03%) and *S. pyogenes* 4(1.35%). Gram positive cocci were susceptible to Linezolid(98.50%) and Vancomycin(93.5%). w Out of 48 cases of Staph aureus, 27(56.25%) were MRSA.

Conclusion: This study will guide the clinician in choosing appropriate antibiotics for better treatment and preventing emergence of the antibiotic resistance.

Keywords: Pyogenic Infections, Antibiotics, MRSA, CLSI,

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 agents commonly known as pus² The most

common organism involved in postoperative infections is *S. aureus*. Surgical procedures in the colorectal or other lower gastro intestinal areas have the highest incidence of post operative infections because of the presence of intestinal flora. These infections are most likely to be caused by enteric gram-negative bacteria, anaerobes, and enterococci⁵. Nosocomial infections are usually caused by coagulase positive and coagulase negative staphylococci. The emergence of drug-resistant organisms in both hospitals and the community is a major concern. Surveillance studies have provided important information about changes in the spectrum of microbial pathogens and trends in the antimicrobial resistance patterns in nosocomial and community acquired infections and continued monitoring of antimicrobial resistance patterns in hospitals is essential to guide effective empirical therapy⁷

Material And Method

The present study was conducted at Microbiology department, JLN Medical College, Ajmer. The study was conducted from December 2018 to June 2019. The study was approved by Institutional Ethical Committee.

Acceptance criteria Pus samples were collected aseptically by sterile aspiration in sterile containers and were accepted for this study.

Rejection criteria Pus samples were received in unsterile containers and with containers soaked with pus due to improper closure of screw cap were rejected.

Entire study was divided into following parts:

1. Sample collection
2. Bacteriological processing and identification
3. Antibigram

Collection of samples Pus samples were collected by using sterile cotton swabs contained in the small screw capped bottles, a firm stopper tube or syringe and

were labeled with the patients name, age, sex, registration no. etc. Then samples were immediately transported to laboratory for culture and identification.

Bacteriological Processing and Identification

The aerobic bacterial isolates were identified by using standard microbiological procedure by colony characteristics, morphology, motility testing and standard biochemical test.

Identification of Gram positive cocci To identify *Staphylococci* following tests were done-

Catalase Test This test differentiates catalase-positive staphylococcal species from catalase-negative streptococcal species.

Coagulase test The test is used to differentiate *Staphylococcus aureus* (positive) from coagulase negative staphylococci (negative).

Aesculin hydrolysis Test This reaction presumptively identifies group D streptococci. The culture of the organism was inoculated on to the slant of the agar medium. It was incubated at 37°C for 48 hours. Enterococci and group D streptococci cause blackening.³⁴

Growth in 6.5% NaCl This test was performed to identify enterococcus. A tube of nutrient broth with 6.5% NaCl was inoculated with 2 or 3 colonies and incubated at 35°C for 3 days. The test was read as positive if the turbidity was observed after 3 days of incubation.

D- Zone test (Inducible Clindamycin Resistance) *S. aureus* isolates were tested for the inducible Clindamycin resistance by double disc diffusion test (D- Zone test). Flattening of the zone (D shaped) of Clindamycin disc towards the disc facing the Erythromycin disc indicates positive D- zone test i.e. presence of inducible Clindamycin resistance.

Antibiogram

For Antibiotic sensitivity pattern, modified Kirby Bauer method was used. It is a type of disc diffusion method. Mueller Hinton agar was used for the antibiotic sensitivity testing. For antimicrobial susceptibility testing CLSI guideline 2018 was used.

Interpretation of zone size-

The diameter of the zone of inhibition was measured and results were prepared. The antimicrobial sensitivity discs manufactured by Himedia laboratories, Mumbai were used. The discs used were as follows-

Antibiotics for Gram positive organisms²⁵

Table-1: Antibiotic symbol and strength for GPC

Antibiotics	Symbol	Strength
Ampicillin	AMP	10 mg
Cotrimoxazole	COT	25µg (23.75/1.25)
Gentamicin	GEN	10 µg
Vancomycin	VA	30 µg
Penicillin-G	P	10 units
Cefoxitin	CX	30 µg
Clindamycin	CD	2 µg
Linezolid	LZ	30 µg
Erythromycin	E	15 µg
Ciprofloxacin	CIP	5 µg

Observations & Results

The present study was carried out with 352 cases of pus samples received from various wards of J.L.N. hospital , Ajmer which were studied in the Department of Microbiology, J.L.N. Medical college, Ajmer. In our study, out of 352 samples, 290(82.39%) samples were culture positive while rest of the 62(17.61%) samples were culture negative .Out of 352 samples maximum no. of samples were received from surgery wards (63%), followed by burn ward (20%) and other were received from medicine ward (4%), ICU (3.4%), CHW. (3.12%), , ORTHO.W (2.5%), ENT.W(2%). Out of 352 cultured smears 175(49.72 %) were Gram-negative bacilli and 110(31 %) Gram-positive cocci. In the present study *K. pneumoniae* was the commonest

organism isolated.. The second commonest organism was *P. aeruginosa* 54(18.30%) followed by *S. aureus* 48(16.28%), CoNS 42 (14.24%), *E. coli* 39(13.22%), *E. aerogenes* 29(9.83%), *Enterococci* 18(6.10%), *P. mirabilis* 6(2.03%) and *S. pyogenes* 4(1.35%).

Table-2: Antibiogram of *S.aureus* & CoNS

Organism		CoNS	<i>S. aureus</i>
CIP	S	17%	10%
	R	83%	90%
CX	S	55%	44%
	R	45%	56%
CD	S	40%	56%
	R	60%	44%
E	S	12%	25%
	R	88%	75%
LZ	S	100%	100%
	R	00%	00%
P	S	19%	17%
	R	81%	83%
COT	S	24%	33%
	R	76%	67%
VA	S	95%	96%
	R	05%	04%

S. aureus and CoNS sensitive to Linezolid and Vancomycin and moderately sensitive to Clindamycin and Cefoxitin but more resistance observed in case of Cotrimoxazole, Ciprofloxacin and Penicillin G.

Table 3:Antibiogram of *Enterococci*

Organism		<i>Enterococci</i>
AMP	S	61 %
	R	39 %
CIP	S	22 %
	R	78 %

GEN	S	33 %
	R	67 %
LZ	S	94%
	R	06%
P	S	39%
	R	61%
VA	S	83%
	R	17%

Enterococci were sensitive to Linezolid 94%, Vancomycin 83%, Ampicillin 61%, Penicillin G 39%, Gentamicin 33% and Ciprofloxacin 22%.

Table 4: Antibigram of *S. pyogenes*

Organism		<i>S. pyogenes</i>
CD	S	75%
	R	25%
E	S	75%
	R	25%
LZ	S	100%
	R	00%
P	S	100%
	R	00%
VA	S	100%
	R	00%

All *Streptococcus pyogenes* were 100% sensitive to Penicillin G, Vancomycin and Linezolid followed by Clindamycin (75%) and Erythromycin (75%)

Table 5: MRSA Vs MSSA

Total no. of <i>S. aureus</i>	MRSA	%	MSSA	%
48	27	56.25%	21	43.75%

The above table shows that the Methicillin resistant *S. aureus* were more (56.25%) compare to Methicillin sensitive *S aureus* (43.75%) which were tested by using Cefoxitin disc (30 µg).

Discussion

In the present study an attempt was made to identify various pus isolates with antibiotic susceptibility testing. The result were compared with other studies and discussed as follows:

Antimicrobial Sensitivity Pattern Resistance to antimicrobial agents has become an emerging problem worldwide because of overuse and irrational prescription of antibiotics. Bacteria adapt various resistance mechanisms as a part of their survival strategy.

Comparison of sensitivity pattern of *S. aureus* with other studies In present study most of *S.aureus* isolates were found sensitive to linezolid (100%) and vancomycin (96%) and resistant to ciprofloxacin (10%) and our study is more or less comparable to Dr. Uma devi s (2017) and Sukumar *etal* (2017).

Comparison of sensitivity pattern of *CoNS* with other studies In present study *CoNS* isolates were found most sensitive to linezolid (100%) and vancomycin (95%) and our study is more or less comparable to Sukumar *etal* (2017)

Comparison of sensitivity pattern of *Enterococci* with other studies In present study *enterococci* most sensitive to linezolid and Vancomycin and more sensitive to Ampicillin and is more or less comparable with Vijeta *et al* (2015) and Sukumar N *etal* (2017). **Comparison of sensitivity pattern of *S. pyogenes* with other study** In present study all *S.pyogenes* were sensitive to linezolid, vancomycin and penicillin-G and comparable with the Roopa C *etal* (2017).

In present study ,Among *S. aureus* 56.25% was found to be MRSA . Shivani K *et al.*, (2016) observed 57.14% and Anupurva *et al.*, (2003) observed 32% of MRSA in their study.

Conclusion

The present study was conducted from DEC. 2018 to JUNE 2019 in the department of microbiology, J.L.N. medical college, Ajmer (Rajasthan). A total 352 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.

1. In present study, culture positivity for pus samples was found to be 82%.
2. In our study Methicillin resistant *Staphylococcus aureus* (MRSA) found more compare to Methicillin sensitive *Staphylococcus aureus* (MSSA).
3. All gram positive cocci showed 100% sensitivity to Linezolid, so this drug remain mainstay in the isolates which are resistant to most of the other antimicrobial drugs.
4. Antimicrobial susceptibility of microorganisms varies from time to time and from place to place. Hence regular monitoring of bacterial susceptibility to antibiotics are essential.
5. Surgical site infections are the major cause of morbidity and mortality of the patients and which causes economical and social loss to patients and their families.

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