RECAST; SPECTRUM OF ANTIBIOTIC ACTIVITY AGAINST GRAM NEGATIVE PYOGENIC MICROORGANISMS IN JAIPUR, RAJASTHAN, INDIA

Dr. Rachna[1], Praveen Kumar Gautam[2] and Dr. Mohammad Suhaib[3 *]

Abstract

Introduction: Crude mortality from transmissible infection in India is about 417% per one lakh persons. Organisms causing pus producing infection is alone a leading cause. Unsatisfactory antibiotic management in country like India has maintain to an increase in antibiotic resistant pyogenic infection caused by bacteria in community and hospital settings.

Material & Methods: This was an observational and retrospective study from July 2020 to December 2020. A total of 145 isolates from pus samples included. Culture was done onto blood enriched agar and MacConkey agar. After culture all agar plates was placed at 37°C for 24 h. Bacterial confirmation was performed according to standard protocol followed in laboratory.

Results: Of the 145 isolates, 51 (35.17%) gram-positive organisms caused pyogenic infections while 94 (64.82%) gram-negative organisms caused pyogenic infections. *Staphylococcus aureus* was the most common isolate, 33 (22.27%) among gram-positive organisms with 21.7% methicillin-resistant staphylococcus aureus, while *Escherichia coli* 30 (20.68%) was the most common isolates among the gram-negative organisms. Third generation cephalosporins did not show significant activity against gram-negative microorganisms. Among the Gram-positive infections, *Staphylococcus aureus* was the predominant organism (74.50%). Among the Gram-negative infections, *Escherichia coli* was predominant organism (31.91%).

Conclusion: Among the Gram-negative microorganisms, 3rd generation cephalosporins show high resistance

Keywords: Pyogenic Infection, wound Infection, Antimicrobial Pattern

[1]Assistant Professor, Department of Medical Laboratory Technology, Sharda School of Allied Health Sciences, Sharda University, Greater Noida, Uttar Pradesh, India

[2] Research Scholar, Department of Microbiology, National Institute of Medical Sciences & Research, NIMS University Rajasthan, Jaipur India

[3*] Assistant Professor, Department of Microbiology, Sharda School of Medical Sciences and Research, Sharda University, Greater Noida, Uttar Pradesh, India

***Corresponding Author:** Dr. Mohammad Suhaib

*Assistant Professor, SSMSR, Sharda University, Email id: 2011sohaib@gmail.com

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Introduction

A thick sticky secretion known as pus is white to yellow fluid containing inactive and regenerating blood cells (WBCs) and surrounding tissues, produced by a wound infection caused by one of the pus-producing bacteria [1-2]. Depending on the causative agent, wound infections can be classified into two types: pyogenic (pus-producing bacteria) and non-purulent. If bacteria form pus, they produce a pyogenic infection, involving local and systemic inflammation, whereas non-pyogenic wound infections are usually caused by atypical mycobacteria, fungi, and viruses [3, 4]. Injuries from abrasions, lacerations, surgical procedures, bites, minor cuts, burns, and lacerations damage intact skin and cause wound infection. The presence of nutrients in the wound attracts bacteria and these bacteria proliferate and release various virulence factors and cause wound infection [5]. Although there are aerobic and anaerobic bacteria that cause wound infections, most cases are caused by aerobic bacteria such as *Staphylococcus aureus, Enterococci, Escherichia. Coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter, Proteus* species [6, 7]. Worldwide, the etiological profile of wound infections is nearly identical with significant variants, but their susceptibility to antibiotic varies by geographic region. Careless and exorbitant use of antibiotics promotes the emergence of antibiotic-resistant organisms, which in turn lead to long hospital stays, massive waste of resources, and serious medical complications. . As a result, the rate of resistant pathogens will increase, which will be a serious problem. Therefore, in this study, we determined the antibiotics activity against pyogenic infection causing microorganisms. In addition, we also investigated the prevalence of methicillin-resistant *Staphylococcus aureus* strains at our hospital setting.

Material & Methods

This is an observational and retrospective study conducted in the Department of Microbiology, National Institute of Medical Sciences & Research, Jaipur, Rajasthan, over a period of 6 months (July 2020 to December 2020). A total of 145 samples collected from pyogenic lesions in IPD and OPD patients in Surgery and Orthopaedics wards at NIMS hospital.

Sample Processing

Cotton swabs placed in screw capped tubes, were immediately sent to Microbiology Laboratory. All samples were inoculated into Blood agar and MacConkey agar. Both the media were incubated for 24 hours at 37^0C. Next day growth was observed.

Isolation, Identification, and Antimicrobial Susceptibility Testing.

The determination was performed using standard procedures including Gram stain, biochemical assays such as catalase, coagulase, indole, methyl red, Voges Prosker, citrate, urease, oxidase disk, hydrolysed bile esculin, etc. Antibiotic susceptibility testing of bacterial strains was performed using the modified Kirby Bauer disc diffusion method on Mueller Hinton agar according to CLSI guidelines [8]. Tobramycin (10 mg), Meropenem (10 mg), Imipenem (10 mg), Gentamicin (10 mg), Levofloxacin (5 mg), Ciprofloxacin (5 mg), Aztreonam (30 mg), Piperacillin-tazobactam (100/10 mg), Ceftriaxone (30 mg), Cefepime (30 mg), Cefuroxin (30 mg), Chloramphenicol (30 mg), Tetracycline (30 mg), Trimethoprim Amikacin (30 mg), Ceftadizime (30 mg) while Ticarcillin-clavulanic acid (75/100 mg) was tested only for Pseudomonas aeruginosa and Acinetobacter spp. For Staphylococcus aureus, Vancomycin (30mg), Teicoplanin (30mg), Gentamycin (10mg), Erythromycin (15mg), Tetracycline (30mg), Doxycycline (30mg), Ciprofloxacin (5mg), Levofloxacin (5mg) , Clindamycin (2mg), Trimethoprim sulfamethoxazole (1.25mg), Chloramphenicol For Enterococci Spp., penicillin (10 units), ampicillin (10mg), vancomycin (30mg), tecoplanin (30mg), ciprofloxacin (5mg), levofloxacin (5mg), erythromycin (5mg), tetracycline (30mg), chloramphenicol (30mg), linezoline (10mg) and high strength gentamicin (120mg). Tests for methicillin-resistant *Staphylococcus aureus* are detected by testing the cefoxitin disc. Antibiotic susceptibility testing was performed on Mueller-Hinton agar plate. A disc of 30 mg cefoxitin was placed and incubated at 37 °C for 24 h. The zone of inhibition of Staphylococcus aureus 21 mm is considered resistant.

Result

Table 1: Distribution of Gram Positive Bacterial Isolates from Tertiary Care Hospital, Jaipur, Rajasthan in Relation to Age and Gender

Age groups	No. of Cases	Male	Female	Staphylococcus *aureus*	Streptococcus Spp.	Enterococcus spp.	Coagulase Negative Staph. auresu
10-20	06	06	-	03	-	02	01
21-40	26	21	05	17	01	02	06
41-60	15	09	06	10	01	02	02
61-80	04	02	02	03	-	-	01
Total	**51**	**38**	**13**	**33**	**02**	**06**	**10**

Table 2: Distribution of Gram Negative Bacterial Isolates from Tertiary Care Hospital, Jaipur, Rajasthan, India in Relation to Age and Gender

Age groups	No. of Cases	Male	Female	E. coli	Klebsiella *pneumoniae*	Pseudomonas *aeuroginosa*	Proteus spp	Acinetobacter spp.	Citrobacter spp.	Burkholderia Spp.
10-20	10	07	03	03	03	03	01	-	-	-
21-40	33	22	11	12	11	06	02	-	01	01
41-60	32	21	11	08	09	11	01	02	01	-
61-80	19	12	07	07	03	03	02	02	01	01
Total	**94**	**62**	**32**	**30**	**26**	**23**	**06**	**04**	**03**	**02**

Table 3: Antibiotic Susceptibility Pattern of Gram Positive Bacteria Isolated from Tertiary Care Hospital, Jaipur, Rajasthan, India

Antibiotics	Sensitive N (%)	Resistant N (%)
VA	47 (92.2%)	04 (7.8%)
TE	46 (90.2%)	05 (9.8%)
DO	39 (76.5%)	12 (23.5%)
P	11 (23.5%)	40 (76.5%)
CD	30 (58.8%)	21 (41.2%)
E	18 (35.3%)	33 (64.7%)
MI	50 (98.0%)	01 (2.0%)
TEI	41 (80.4%)	10 (19.6%)
AK	33 (66.7%)	18 (33.3%)
C	42 (82.4%)	09 (17.6%)
COT	35 (68.6%)	16 (31.4%)
GEN	36 (70.6%)	15(29.4%)
TOB	44 (86.3%)	07(13.7%)
CIP	23 (45.1%)	28 (54.9%)
LE	30 (58.8%)	21 (41.2%)
LZ	49 (96.1%)	02 (3.9%)
TGC	49 (96.1%)	02 (3.9%)
CX	43 (84.3%)	08 (15.7%)

VA- Vancomycin, Te- Tetracycline, DO- Docycycline, P-Penicillin, CD- Clindamycin, E- Erythromycin, MI-Minocycline, Tei- Tecoplanin, AK-Amikacin, C- Chloramphenicol, COT- Trimethoprim sulfamethoxazole, GEN-Gentamicin, TOB- Tobramycin, CIP- Ciprofloxacin, LE- Levofloxacin, LZ- Linezoline, TGC- Tigecycline, CX-Cefoxitin

Table 4: Antibiotic Susceptibility Pattern of Gram Negative Bacteria Isolated from Tertiary Care Hospital, Jaipur, Rajasthan, India

Antibiotics	Sensitive N (%)	Resistant N (%)
TOB	51 (54.3%)	43 (45.7%)
MRP	64 (68.1%)	30 (31.9%)
IPM	53 (56.4%)	41 (43.6%)
GEN	37 (39.4%)	57 (60.6%)
AK	55 (58.5%)	39 (41.5%)
LE	43 (45.7%)	51 (54.3%)
CIP	44 (46.8%)	50 (53.3%)
AT	43 (45.7%)	51 (54.3%)
PIT	58 (61.7%)	36 (38.2%)
CTR	23 (24.5%)	71 (75.5%)
CPM	36 (38.3%)	58 (61.7%)
CTX	22 (23.4%)	72 (76.6%)
CAZ	31 (32.9%)	63 (67.0%)
CFM	21 (22.3%)	73 (77.7%)
C	59 (62.8%)	35 (37.2%)
TE	41 (43.6%)	53 (56.4%)
COT	32 (32.9%)	63 (67.0%)
AMP	28 (29.8%)	66 (70.2%)
CXM	23 (24.5%)	71 (75.5%)

TOB- Tobramycin, MRP- Meropenem, IPM- Imipenem, GEN- Gentamicin, AK- Amikacin, LE- Levofloxacin, CIP- Ciprofloxacin, AT- Aztronem, PIT- Piperacillin-tazobactam, CTR- Ceftraxone, CPM- Cefepime, CTX- Cefotaxime, CAZ- Ceftadizime, CXM- Cefuroxime, TCC- Ticarcillin- clavulanic acid, CX- Cefoxitin, MI- Minocycline, C- Chloramphenocol, TE- Tetracycline, COT- Trimethoprim sulfamethoxazole, AMP- ampicillin

Discussion

A total of 145 pus swab samples were received for culture and sensitivity over a period of six months in the MICROBIOLOGY DEPARTMENT of the National Institute of Medical Sciences and Research.

On the basis of conventional bacterial identification techniques such as gram's stain, culture characteristics, and biochemical reactions, eleven different bacterial pathogens were identified. Out of these 145 isolates, 94 (65%) were gram negative bacilli and 51 (35%) were gram's positive cocci. In the present study, gram's-negative pyogenic bacterial infections are high. Similar findings were also present in other studies. [9]

Staphylococcus aureus was the most common isolate at 33 (22.27%), followed by *Escherichia coli* 30 (20.68%), *Klebsiella pneumoniae* 26 (17.93%), *Pseudomonas aeruginosa* 23 (15.68%), Coagulase negative *Staphylococcus aureus* 10 (6.89%), *Proteus* and *Enterococcus* 06 (4.13%), *Acinetobacter Spp*. 04 (2.75%), Citrobacter spp. 03 (2.06%), *Burkholderia and Streptococcus pyogenes* 02 (1.37%).

Staphylococcus aureus was the most common organism in the current study, followed by Coagulase negative *Staphylococcus aureus*, *Enterococcus*, and *Streptococcus pyogenes* among gram positive organisms. A study conducted by the Indian Council of Medical Research antimicrobial resistance surveillance network also reported *Staphylococcus aureus* to be the most prevalent isolated organism from patients with skin and soft tissue infections (73.7%), but these findings are higher than in the present study. Other studies also supported these findings [11, 12, 13, 14, 15]. The higher prevalence of *staphylococcus aureus* may be due to inanimate objects, health care workers, and other patients having a high carrier rate. Further, due to the presence of *Staphylococcus aureus* as a normal flora of the human body, endogenous infections are also possible. The prevalence of methicillin-resistant *staphylococcus aureus* (MRSA) in the present study was 21.7%. The Indian network for surveillance of antimicrobial resistance (INSAR) reported a 40% prevalence of MRSA [16]. The prevalence in the present study was less than reported by INSAR. This may be due to the smaller sample size in the present study. Among gram-positive pathogens, *Staphylococcus aureus* was commonly isolated, followed by CONS and *Enterococci*, which correlates with the study done by Kumar PH et al. [15]. Among gram-negative isolates, *Escherichia coli* was the most prevalent in the present study. A study from the same state supported these findings [17, 18]. Another study from India also showed *Escherichia coli* was the most prevalent organism among gram-negative isolates.

The antimicrobial patterns of different gram-positive isolates are shown in Table 3. The most sensitive antibiotics for S*taphylococcus aureus* were Linezoline, tetracycline, and vancomycin. However, 3.03%, 30.03%, and 6.06% resistance was also observed for Linezoline, Tetracycline,

and Vancomycin, respectively. Bankar et al. showed 97.1% sensitivity and 2.9% resistance to linezolid [19]. Another study done by Verma P showed 100% sensitivity to vancomycin and 92.5% sensitivity to linezoline [20]. In the present study, the linezolid resistance rate was very much similar. Because of the availability of oral formulation, local practitioners are incorrectly using linezolid as first-line therapy in infections.

In the present study, 3rd generation cephalosporin, Trimethoprim-sulfamethoxazole, ampicillin resistance was much higher in *Escherichia coli* than in *Klebsiella* spp. It was similar to Rugina et al. [21]. It may be due to the higher prevalence of *E. coli* infection compared to *Klebsiella* infection in our hospital locality and, thereby, more antibiotic pressure on the *E. coli* strain. *Citrobacter* species showed resistance to 3rd generation cephalosporin but high sensitivity to Tobramycin, Meropenem, Piperacillin-tazobactum, Cefoxitin, and chloramphenicol that was similar to Rugina et al. [21]. Except for Aztreonem, tetracycline, and ampicillin, all antibiotics were effective against *Proteus species.* Overall, all gram negative bacilli showed high resistance to 3rd generation cephalosporin like Duggal et al [22]. Jamatia and colleagues [23] Rozina and colleagues [24-25] Balan et al.

Conclusion

This study showed that the most common organism causing pyogenic infection was *Staphylococcus aureus,* followed by *Escherichia coli, Klebsiella species, Pseudomonas aeruginosa*, and Coagulase negative *Staphylococcus aureus*, *Proteus* and *Enterococcus, Acinetobacter species, Citrobacter species, Burkholderia species*, and *Streptococcus pyogenes* are all examples of pathogens. Linezolid, tetracycline, Gentamycin, and piperacillin-tazobactam could be used as empirical therapy for treating these infections. Most of the 3rd generation cephalosporins have become resistant, so they should not be used to treat pyogenic infections in hospitals. However, continuous surveillance of antibiotic sensitivity patterns needs to be done to determine the true incidence of antibiotic resistance in the community and prevent outbreaks by implementing proper antibiotic policy.

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