International Journal of Biomedical Research
ISSN: 0976-9633 (Online); 2455-0566 (Print)
Journal DOI: https://doi.org/10.7439/ijbr
CODEN: IJBRFA

Original Research Article

Bacteriological profile and antibiogram of pus isolates in patients of tertiary care hospital in Central India

Rahangdale Hina[1], Supare Sundaram[*2], Rahangdale Vaishali[1], Kokate Sandeep[3] and Shrikhande Sunanda[4]

[1]Assistant Professor, [2]Senior Resident, [3]Associate Professor, [4]Professor and Head,
Department of Microbiology, Government Medical College (GMC), Nagpur, Maharashtra, India

Abstract

Introduction: Pyogenic wound infections caused by various pathogens like bacteria, fungi, viruses and parasites are difficult to treat because of change in antimicrobial susceptibility pattern and lack of awareness about judicious use of antimicrobials. Hence it is important to study the pathogens causing the infections and its antibiotic susceptibility pattern for proper management of the patients.

Material & methods: This observational study was conducted for a period of 6 months. A total of 772 pus samples were collected and identified by standard microbiological procedures and subjected to antimicrobial susceptibility testing as per CLSI 2018 guidelines. Staphylococcal isolates were screened for methicillin and inducible clindamycin resistance. Gram negative isolates were screened for extended spectrum ß-lactamase resistance and metallo ß- lactamase resistance.

Results and discussion: Out of 772 pus samples, 471 (61.01%) organisms were isolated which included 135 (28.67 %) gram positive cocci,318 (67.51%) gram negative bacilli and 18 (3.82 %) were *Candida* spp. *Staphylococcus aureus* was predominant bacterial isolates accounting for 120 (25.48%) followed by *E. coli* 105(22.29%), *Klebsiella pneumoniae* 72(15.28%), *Acinetobacter baumanii* 51(10.82%) and *Pseudomonas aeruginosa* 45(9.55%).

Conclusion: This study emphasizes the need to know the common organisms isolated from pus samples and their antimicrobial susceptibility pattern which further helps in guiding empirical treatment of patients and reducing the spread of resistant strain in community.

Keywords: Pus isolates, Antimicrobial susceptibility, MRSA, ESBL.

***Correspondence Info:**
Dr. Sundaram Supare
Senior Resident,
Department of Microbiology,
Government Medical College (GMC),
Nagpur, Maharashtra, India

***Article History:**
Received: 02/10/2019
Revised: 27/10/2019
Accepted: 28/10/2019
DOI: https://doi.org/10.7439/ijbr.v10i10.5291

QR Code

How to cite: Rahangdale H, Supare S, Rahangdale V, Kokate S and Shrikhande S. Bacteriological profile and antibiogram of pus isolates in patients of tertiary care hospital in Central India. *International Journal of Biomedical Research* 2019; 10(10): e5291. DOI: 10.7439/ijbr.v10i10.5291 Available from: https://ssjournals.com/index.php/ijbr/article/view/5291

1. Introduction

Pyogenic infections of human skin and soft tissue infections caused during or after trauma, burn injuries and surgical procedures, are characterized by involvement of different microorganisms leading to formation of pus.[1] Different studies have shown that aerobic bacteria are majority of isolates causing pyogenic infections. The most common pyogenic organisms include gram positive cocci like *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Enterococcus* spp. and gram negative bacilli like *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp. and *Pseudomonas* spp.[2] Even though the bacteriological profile of pus samples in many studies remain the same, the antibiotic resistance pattern of these isolates have shown a lot of variations.[3,4] Irrational use and inadequate dose regimen of antimicrobial has led to emergence of multi-drug resistance, making treatment difficult.[5] This has resulted in increase in morbidity, hospital stay and higher health care expenditure. In addition antimicrobial resistance hampers the control of infectious diseases by reducing the effectiveness of treatment. Thus the patients remain infectious for a long time increasing the risk of spreading resistant microorganisms to others. [6]

Continuous monitoring of bacteriological profile from pus samples and its sensitivity would highlight variations in resistance pattern of the organisms. [7] Thus, this study was aimed at evaluating bacteriological profile and antimicrobial resistance pattern in pyogenic infections.

2. Material and methods

This observational study was conducted from June 2018 to December 2018.

1) Isolate pool: A total of 772 non replicate clinical and invasive isolates were obtained from pus samples derived from superficial wounds and deep seated abscesses using standard bacteriological techniques and identified by conventional methods. [8]

2) Antimicrobial susceptibility testing: Antimicrobial susceptibility testing of these clinical isolates were determined by Kirby Bauer disc diffusion method and analyzed according to CLSI 2018 guidelines. *Staphylococcus aureus* were screened for methicillin and inducible clindamycin resistance. Extended spectrum ß-lactamases (ESBL) resistance and metallo ß- lactamases (MBL) production were detected in gram negative bacterial isolates. [9]

i) ESBL: Phenotypic confirmatory disk diffusion test- Combined disk diffusion method was ceftazidime (30µg) disc alone and in combination with clavulanic acid disc (30/10µg). The test organism was inoculated on Muller Hinton Agar plate; discs were placed and incubated overnight at 37^0C. [9]

Interpretation: Isolates showing zone of inhibition of ceftazidime plus clavulanic acid disc ≥ 5mm than those of ceftazidime disc alone was interpreted as ESBL producers.

ii) MBL production: [10] The Disk Potentiation Test - A lawn culture of the test strain was done on Mueller Hinton agar plates. Two 10µg imipenem disks were placed on inoculated plates wide apart and 10µl of 50mM zinc sulphate solution was added to each of the imipenem disks. Then 10µl of 0.5M EDTA solution was added to one imipenem disk. The inhibition zones of imipenem and imipenem -EDTA disks were compared after overnight incubation at 37^0C.

Interpretation: An increase in zone size ≥7mm around the Imipenem-EDTA disk as compared to the imipenem only disk was recorded to be MBL producers.

iii) MRSA: *Staphylococcus* species were screened for methicillin resistance *Staphylococcus aureus* (MRSA) by using cefoxitin disc (30ug)

Interpretation: Zone size ≤ 21mm shows strain as resistant to cefoxitin indicating as MRSA.

iv) Inducible clindamycin resistance:[9] D-test - Erythromycin (15ug/ disc) was placed at a distance of 15mm (edge to edge) from clindamycin (2ug/disc) on Muller Hinton agar plate, previously inoculated with bacterial suspension equivalent to 0.5 McFarland standards and incubated overnight at 37℃.

Interpretation: Flattening of zone (D-shaped) around clindamycin in the area between the two discs, indicate inducible clindamycin resistance.

v) Quality control [9]

a) Quality of Muller Hinton agar was checked for sterility and by its ability to support the growth of ATCC 25923 *S. aureus*.

b) Quality of ceftazidime, ceftazidime with clavulanic acid, imipenem, imipenem-EDTA disc was tested with *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

c) Quality of discs of cefoxitin (30ug), erythromycin (15ug) and clindamycin (2ug) disc were checked by an appropriate zone of inhibition using ATCC 25923 *S. aureus.*

3. Results & discussion

Out of total 772 pus samples received in microbiology laboratory, 471 (61.01%) organisms were isolated. No organism was isolated in 262 processed pus samples. Thirty nine (5.05%) samples showed polymicrobial growth. Out of 471 isolates, 135 (28.67%) were gram positive cocci, 318 (67.51%) gram negative bacilli and 18 (3.82%) *Candida* spp.

Table 1: Distribution of organisms isolated from pus samples: (n=471)

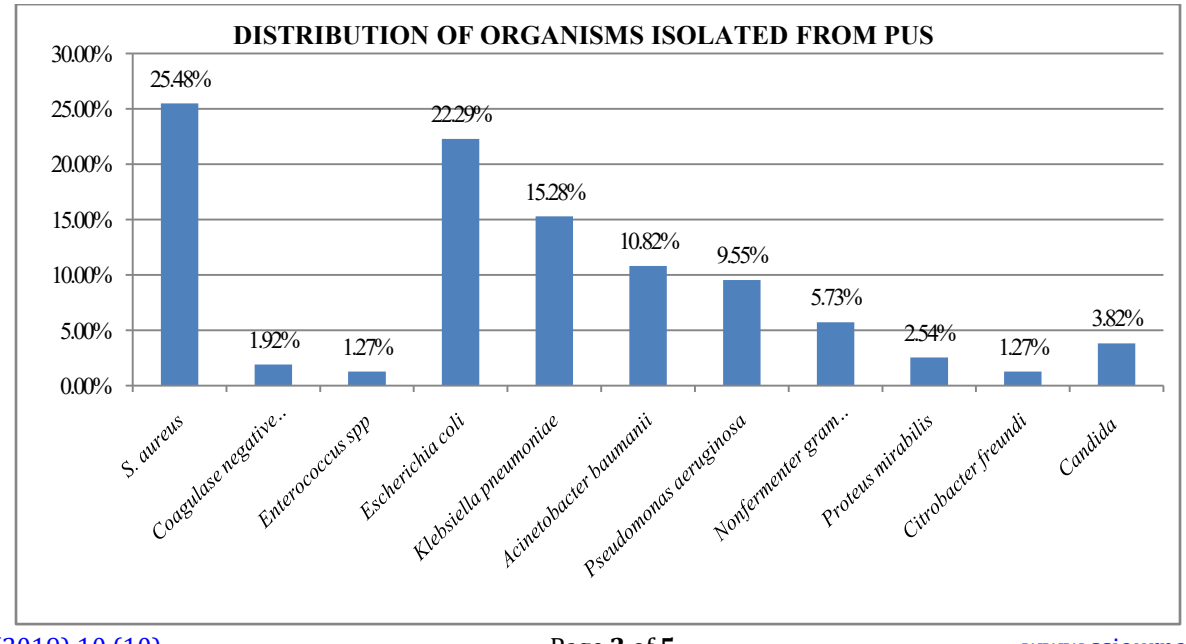

Table 2: Antimicrobial resistance pattern of gram positive cocci

Antibiotic	*Staphylococcus aureus* n=120 (%)	*CONS* n=9 (%)	*Enterococcus* species n=6 (%)
Erythromycin	30(25)	3(33.33)	2(33.33)
Clindamycin	27(22.5)	0	2(33.33)
Cefoxitin	63(52.5)	3(33.33)	-
Penicillin	117(97.5)	9(100)	5(83.33)
Tetracycline	3(2.5)	0	2(33.33)
Ciprofloxacin	108(90)	0	2(33.33)
Gentamicin	21(17.5,)	3(33.33)	4(66.66)

Table 3: Antibiotic resistance pattern of gram negative bacilli

Antibiotic	*E. .coli* n=105 (%)	*K. pneumoniae* n=72 (%)	*Proteus* species n=12(%)	*Citrobacter* spp n=6 (%)	*A. baumanii* n=51(%)	*P. aeruginosa* n=45(%)	Non-fermenter gram negative bacteria n=27(%)
Gentamicin	15(14.29)	33(45.83)	3(25)	3(50)	39(16.47)	24(53.33)	15(55.56)
Tobramycin	24(22.86)	36(50)	6(50)	3(50)	20(39.22)	15(33.33)	15(55.56)
Amikacin	27(25.71)	39(54.17)	6(50)	2(33.33)	25(49.02)	21(46.67)	18(66.66)
Piperacilin tazobactum	45(42.86)	39(54.17)	3(25)	2 (33.33)	14(27.45)	10(22.22)	21(77.77)
Cefepime	90(85.71)	63(87.5)	9(75)	3 (50)	42(82.35)	27(60)	18(66.67)
Cefoxitin	69(65.71)	60(83.33)	6(50)	6(100)	36(70.59)	-	-
Ceftazidime	81(77.14)	60(83.33)	6(50)	3 (50)	41(80.39)	30(60.67)	18(66.67)
Ceftriaxone	78(74.29)	66(91.67)	9(75)	3(50)	-	-	-
Ciprofloxacin	66(62.86)	60(83.33)	12(100)	6(100)	42(82.35)	24(53.33)	18(66.67)
Imipenem	36(34.29)	39(54.18)	3(25)	-	21(41.18)	18(40)	3(11.11)

Table 4: Distribution of ESBL & MBL in gram negative bacilli

Organism	ESBL (%)	MBL (%)
E. coli	9(2.83)	3(0.94)
Klebsiella pneumonia	3(0.94)	9(2.83)
Acinetobacter baumanii	0	6(1.88)
Pseudomonas aeruginosa	0	3(0.94)

In the present study, among 471 culture positive samples, *Staphylococcus aureus* 120(25.48%) was predominant bacterial isolates. Roopa C *et al* (16.47%) & Muluye *et al* (63.9%), Subha *et al* (25.32%)[11-13] showed the same finding of predominant isolation of *Staphylococcus aureus* whereas Trojan *et al* and Gomatheswari *et al* [4,7] reported gram negative isolates as predominant organism isolated from pus samples.

In the present study, maximum numbers of isolates of *Staphylococcus aureus* were resistant to penicillin (97.5%) followed by ciprofloxacin (90%) and cefoxitin (52.5%). Methicillin resistant *Staphylococcus aureus* (MRSA) was found to be 32.5%in present study. Various studies from India also reported the MRSA rate to be ranging from 20-45%. [14, 15-17] In the present study, inducible clindamycin resistance in *S. aureus* (ICR) was 27.5%. This is in concordance with study of Kalbhor *et al* (28.41%) and Reddy *et al* (26.53%). [18, 19]

Staphylococcus aureus strains showing both MRSA & ICR were found to be 20%. Similar results were shown by Kalbhor *et al* (24.62%), More *et al* (22.58%), Mazhi *et al* (24.8%) whereas Jarajreh *et al* (76.7%) reported higher percentage. [18,20-22]

Staphylococcus aureus is one of the most common organisms of skin and soft tissue abscess. It also causes pneumonia, toxic shock syndrome, exfoliative skin disease and enteritis. It is a colonizer of skin, nail, nares and spread via physical contact and aerosols. [6] Improper use of antibiotics has led to drug resistant strains like MRSA, ICR, thus judicious use of these antibiotics is the need of hour.

Among 318 (67.51%) gram negative isolates, *E. coli* was a predominant isolate 105(22.29%) followed by *Klebsiella pneumonia* 72(15.28%), *Acinetobacter baumanii* 51(10.82%) and *Pseudomonas aeruginosa* 45(9.55%). This was in agreement with study of Roopa C *et al* (19.14%) [11].

Antibiogram results from present study showed that *E. coli* were more resistant to cephalosporins and ciprofloxacin while being less resistant to amikacin, gentamicin whereas *Klebsiella pneumonia* and *Acinetobacter baumanii* were resistant to most of the antibiotics. *Pseudomonas* spp. was found to be more resistant to cephalosporin and ciprofloxacin while it was least resistant to piperacillin-tazobactum. In present study, *Proteus* spp. and *Citrobacter* spp. were susceptible to most of the antibiotics. These finding of antibiogram coincides with the observations of Roopa *et al*[11].

In the present study, about 3.77% isolates were ESBL producers. There is high incidence of ESBL isolates in *E. coli* (2.83%) followed by *Klebsiella pneumoniae* (0.94%). Previous studies from India have reported the prevalence of the ESBL producers to be 6.6% to 53%.

Subha *et al* reported 6.6% ESBL producers among *Klebsiella pneumoniae* from children, Menon *et al* (20%), Babypadmini *et al* (40.3%), Rodrigues *et al* (53%), Singhal *et al* (64%) ESBL production in their study cohort. [23-27]

Total MBL isolates were 6.6%, amongst which *Klebsiella* spp. has shown higher incidence of 2.83% than *Acinetobacter* spp, *E. coli*, and *Pseudomonas* species as shown in table 4.

The differences in prevalence and antibiotic resistance patterns of pyogenic bacterial isolates could be due to variations in geographic areas and different climatic conditions. Existence of high drug resistance to multiple antibiotics in isolates from pus samples in present study and several other related reports points towards incomplete treatment schedules, antibiotics misuse, lack of regional antibiogram data, and limited knowledge about multidrug-resistant isolates among clinicians. Updating knowledge of antimicrobial susceptibility profiles of clinical isolates will help in designing the most appropriate dose-regimen and treatment schedule against wound infections.

4. Conclusion

Hence this study underlines the necessity to update the knowledge of antibiogram of the pus isolates to provide the most appropriate dose regimen and to limit the emergence of drug resistant strains.

References

[1]. Scalise A, Bianchi A, Tartaglione C, Bolletta E, Pierangeli M, Torresetti M, Marazzi M, Di Benedetto G. Microenvironment and microbiology of skin wounds: the role of bacterial biofilms and related factors. *Semin Vasc Surg.* 2015; 28(3-4):151-9.

[2]. Biradar A, Farooqui F, Prakash R, Khaqri S, Itagi I. Aerobic bacteriological profile with antibiogram of pus isolates. *Indian J Microbiol Res* 2016;3(3):245-9.

[3]. Duggal S, Khatri P, Parihar R, Arora R. Antibiogram of various bacterial isolates from pus samples in a tertiary care centre in Rajasthan. *International Journal of Science and Research*. 2015;4(5):1580-4.

[4]. Trojan R., Razdan L, Singh N. Antibiotic Susceptibility Patterns of Bacterial Isolates from Pus Samples in a Tertiary Care Hospital of Punjab, India. *International Journal of Microbiology*. 2016; article ID 9302692, 4 pages, 2016.

[5]. Krishnamurthy S, Sajjan A, Swetha G, Shalini S. Characterization and resistance pattern of bacterial isolates from pus samples in a tertiary care hospital, Karimnagar. *Trop J Pathol Microbiol*. 2016; (2): 49-54.

[6]. Bankar N, Wankhade A, Bramhane R, Hathiwala R, Chandi D. Bacteriological Profile of pus / wound swab and antimicrobial susceptibility of *Staphylococcus aureus*. Isolated from of pus & wound swab of indoor patients of tertiary care hospital in Durg, Chhattisgarh, India. *International Journal of Innovative Research in Medical Science*. 2018;3(4): 1976 -80

[7]. Gomatheswari N, Jeyamurugan T. Bacteriological Profile and the Antibiotic Susceptibility Pattern of Microorganisms Isolated from Pus/Wound Swab Isolates in Patients Attending a Tertiary Care Hospital in South India. *Int. J. Curr. Microbiol. App. Sci.* 2017; 6(10): 1405-13.

[8]. Colle JG, Mikes RS, Watt B. Tests for identification of bacteria. In: Collee J G, Marmion BP, Fraser AG, Simmons A, editors. Mackie and MacCartney Practical Medical Microbiology.14thed. London: Churchil Livingston; 1996b:131 -49.

[9]. Cockerill FR, Patel JB, Alder J et al. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100, Wayne, PA: Clinical and Laboratory Standards Institute. 2018.

[10]. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo β-lactamase producing clinical isolates of Pseudomonas species &Acinetobacter species. *J Clin Microbiol* 2002; 40: 3798-801.

[11]. Roopa C, Deepali V. Pus culture isolates and their antibiotic sensitivity at a Tertiary Care Hospital in Hyderabad Karnataka Region**.** *International Journal of Medical Microbiology and Tropical Diseases*. 2017;3(4):140-5

[12]. Muluye D, Wondimeneh Y, Ferede G, Nega T, Adane K, Biadgo B, et al. Bacterial isolates and their antibiotic susceptibility patterns among patients with pus and/or wound discharge at Gondar university hospital. *BMC Research Notes.* 2014; 7:619.

[13]. Subha M, Srinivasagam M. Microbial Profile and Antimicrobial Susceptibility Pattern of Pus Culture Isolates from a Teaching Tertiary Care Hospital, South India. *Int. J. Curr. Microbiol. App. Sci.* 2018; 7(04): 1149-53.

[14]. Mokta K, Verma S, Chauhan D, Ganju S, Singh D, Kanga A, Kumari A, Mehta V. Inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* from Sub Himalayan Region of India. *J Clin Diagn Res* 2015; 9:20-3.

[15]. Gupta V, Datta P, Rani H, Chander J. Inducible clindamycin resistance in *Staphylococcus aureus*: a study from north India. *J Postgrad Med* 2009: 55; 176–9.

[16]. Deotale V, Mendiratta DK, Raut U, Narang P. Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. *Indian J Med Microbiol*.2010; 28:124-6.

[17]. Gade N, Qazi MS. Inducible clindamycin resistance among *Staphylococcus aureus* isolates. *Indian Journal of Basic & Applied Medical Research* 2013; 2(8): 961- 7.

[18]. Kalbhor P, Wanjare V, Shrikhande S. Detection of inducible and constitutive clindamycin resistance among clinical isolates of *Staphylococcus aureus* in a tertiary care hospital. 2018:8(9); 70-2.

[19]. Reddy M, Hima C, Bindu M, Soumendranath M, Kanta RC, Kapur I. Prevalence of inducible clindamycin resistance in *Staphylococcus aureus* from clinical samples: a study from a teaching hospital in Andhra Pradesh, India. *Int J Curr Microbiol App Sci* 2014; 3: 402-9.

[20]. More S, Kasturi, Rathod V, Sinha R, Khan S. Inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* in a tertiary care hospital, Nanded, Maharashtra. *Int. J. Current Microbiol. App. Sci.* 2017: 6(4); 1232-9.

[21]. Majhi S, Dash M, Mohapatra D, Mohapatra A, Chayani N. Detection of inducible and constitutive clindamycin resistance among *Staphylococcus aureus* isolates in a tertiary care hospital, Eastern India. *Avicenna J Med*. 2016; 6(3):75-80.

[22]. Jarajreh D, Aqel A, Alzoubi H, Al-Zereini W. Prevalence of inducible clindamycin resistance in methicillin resistant *Staphylococcus aureus*: the first study in Jordan. *J Infect Dev Ctries*. 2017; 11:350-4.

[23]. Subha A, Ananthan S. Extended-spectrum β-lactamase (ESBL) mediated resistance to the third generation cephalosporins among *Klebsiella pneumoniae* in Chennai. *Indian J Med Microbiol* 2002; 20:92-5.

[24]. Menon T, Bindu D, Kumar C, S Nalini, Thirunarayan M A. Comparison of double disc and three dimensional methods to screen for ESBL producers in a tertiary care hospital. *Indian Journal of Medical Microbiology* 2006; 24:117-120.

[25]. Babypadmini S, Appalaraju B. Extended-spectrum β-lactamases in the urinary isolates of Escherichia coli and Klebsiella pneumoniae – prevalence and susceptibility pattern in a tertiary care hospital. *Indian J Med Microbiol* 2004; 22(3): 172-4.

[26]. Rodrigues C, Joshi P, Jani SH, Alphonse M, Radhakrishnan R, et al. Detection of β-lactamases in nosocomial gram negative clinical isolates. *Indian J Med Microbiol* 2004; 22(4):247-50.

[27]. Singhal S, Mathur T, Khan S, Upadhyay D, Chugh S, Gaind R et al. Evaluation of the methods for Amp C β-lactamase in gram negative clinical isolates from tertiary care hospitals. *Indian J Med Microbiol* 2005; 23(2):120-24.