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BACTERIOLOGICAL PROFILE OF PUS SAMPLES AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL, RANCHI.

Microbiology

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ABSTRACT

Background: Pyogenic infections can be caused by various microorganisms and mixed infections are common which require antibiotic therapy. The inappropriate use of antibiotics has resulted in development of antibiotic resistance. The bacteriological profile may remain same, but antibiotic susceptibility pattern varies. Hence the study was conducted to know bacteriological profile of pus samples and their antibiotic susceptibility pattern in patient attending RIMS, Ranchi.
Materials and Methods: This retrospective study was conducted over the period of one year from 1st November 2020-30 th April 2021at Microbiology laboratory of Rajendra Institute of Medical Sciences (RIMS), Ranchi to find the prevalence of microorganism and antimicrobial resistance . The samples were cultured on Blood agar and MacConkey agar. After aerobic incubation at 37˚C for 18-24 hrs, organisms were identified by standard methods and antibiotic susceptibility was tested by Kirby Bauer disc diffusion method. Methicillin resistant Staphylococcus aureus (MRSA) was tested by Cefoxitin 30 µg disc and ESBL by combined disk.
Results: The overall pus culture done in 6 months were 1025, out of which growth was seen in 642 cultures. Staphylococcus aureus 193(30.06%) was most common gram positive isolate and Klebsiella pneumonia 140(21.81%),was most common gram negative isolate. ESBL positivity was seen in 237(57.80%) and MRSA in 77(39.90%)Staphylococcus aureus isolates. Most of gram negative isolates were susceptible to piperacillin / tazobactum 342(83.41%)and meropenem 349(85.12%) and in gram positive all the isolates were susceptible to vancomycin 193(100%) and linezolid 193(100%).
Conclusion: To combat resistance,irrational use of antibiotics should be avoided. Also regular surveillance helps in implementing better therapeutic strategies to reduce morbidity and mortality. It is important for a clinician to send all the pus sample for microbiological analysis and their antibiogram before putting cases on antibiotics so that emergence of drug resistance can be minimized.

KEYWORDS

Antibiotic sensitivity test, Methicillin resistant Staphylococcus aureus (MRSA), Extended-Spectrum Beta-Lactamase (ESBL),Coagulase negative Staphylococcus aureus(CONS).

INTRODUCTION

Pyogenic infection causes local inflammation, formation of pus, generally caused by one of the pyogenic bacteria, which result in the aggregation of dead leukocytes as well as pyogenic bacteria commonly known as pus.[1] Wounds are result of loss of intact skin due to injury caused by external forces such as surgical wounds, burns, bites, abrasions, minor cuts and more severe traumatic wounds such as lacerations and crush or gunshot injuries such discontinuity in skin is good environment for microbial colonization as there is presence of moisture, warmth and nutrition for their growth. Colonization with proliferation of bacterial flora may lead to wound infection which may be serious even sometimes lead to death. [2]

The occurrence of wound infections depends on various factors like condition of wound, microbial load and the host defense mechanisms. [3] The overall incidence of wound sepsis in India is from 10% to 33%. [4] The infecting pathogens not only differ from country to country, but also vary from one hospital to another within the same country. [5] It is caused by bacteria, virus, fungi and protozoa and in many cases there is a mixed infection with more than one bacterial species. [6] The most common causative agent includes Staphylococcus aureus which account for 20-40%. 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. [7,8]

Since the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in 1960, there have been reports of increasing rate of infection by MRSA and this superbug has established itself as the common cause of nosocomial as well as community acquired infections [4]

Inadvertent and inappropriate use of antibiotic leads to emergence of drug resistant pathogen that in turn leads to long hospital stay, huge loss of resource and serious medical complication. In long hospital stay the patient can transfer drug resistant bacteria to other patients or relatives or even to care providers. So, background knowledge of local microbial profile and antibiogram of isolates causing wound infection is encouraging for clinician to treat wound infection empirically. Considering paucity of data in this regard in Jharkhand we have conducted a research to find out microbial profile and antibiogram of pus isolates at RIMS, Ranchi.

MATERIALS AND METHODS

A retrospective study was conducted at Rajendra Institute of Medical Sciences,Ranchi from November 2020 to April 2021. Pus samples were aseptically collected using sterile swab in a test tube and inoculated on to blood agar and MacConkey agar. Plates were incubated at 37◦C for 24 hours. Organisms were identified by series of biochemical reactions standard following standard procedures.

Antimicrobial susceptibility testing was performed using Muller-Hinton agar plates by disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines. 15 The isolates were tested against ampicillin (10 µg), amoxyclav (20/10µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), Cefotaxime (30 µg), Meropenem (10 µg) and Piperacillin – Tazobactum (100/10µg). For gram-positive isolates, Cotrimoxazole (1.25µg /23.75µg), Erythromycin (5µg), Clindamycin (2µg), Chloramphenicol (30µg), Tetracycline (30µg), Linezolid (30µg), Vancomycin(30µg). For Enterococci high level gentamicin (HLG) was used.

ESBL was detected by combined disk test. This was performed by phenotypic confirmatory test as per the recommendations of CLSI. The ceftazidime (30 µg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 µg discs) were used. An increase of ≥ 5mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be ESBL producer.

Methicillin resistant Staphylococcus aureus (MRSA) was detected by Cefoxitin disc diffusion test. Lawn culture was done onto Mueller–Hinton agar plate. A 30 µg cefoxitin disc was placed and incubated at 37◦C for 24 hrs. The zone of inhibition of S. aureus ≤ 21 mm was considered as methicillin resistant.

E. coli ATCC 25922, Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATCC 27853 and E. faecalis ATCC 29212 strains were used for quality control of biochemical test and antibiotic sensitivity test.Statistical analysis Analysis was done using MS Excel.

RESULTS

A total number of 1025 patients presented with wound infection or pus

were recruited for this study. Among 1025 patients majority were in the age group of 20 to 40 years which was 432(42.15%) 226(22.05%) cases respectively.

Table 1: Age and Gender Distribution of Study Population (n=1025)

Age group	Male	Female	Total
Less than 20	120(20.87%)	106(23.56%)	226(22.05%)
20-40	231(40.17%)	201(44.47%)	432(42.15%)
40-60	201(34.96%)	134(29.78%)	335(32.68%)
More than 60	23(4%)	9(5%)	32(3.12%)
Total	575	450	1025

Interestingly male was predominant than female which was 575(56.10%) cases and 450(43.90%) cases respectively.

Table 2: Culture Positivity of Study Population (n=1025)

Culture	Frequency	Percentage
No growth	383	37.37%
Growth	642	62.63%
Total	1025	100%

Among the male and female group 20 to 40 Years was the most common age group which was 231(40.17%) cases and 201(44.47%) cases respectively (Table 1). Out of 1025 cases aerobic culture was positive in majority cases which were 642(62.63%) cases and the rest of 382(37.37%) cases were growth negative. Therefore culture positive was more than no growth which was shown in this result and reflected the laboratory authenticity (Table 2).

Table 3: Rate of Isolated Bacteria after Aerobic Culture (n=1025)

Bacterial isolates	Number	Percentage (%)
Staphylococcus aureus	193	30.06
CONS	22	3.43
Enterococcus species	17	2.65
Pseudomonas aeruginosa	87	13.55
Klebsiella pneumoniae	140	21.80
Acinetobacter baumanii	72	11.21
Proteus species	40	6.23
Escherichia coli	71	11.06
Total	642	100

Staphylococcus aureus was the most common isolated bacteria from pus which was 193(30.06%) isolates followed by *Klebsiella , Pseudomonas, E coli, Acinetobacter species* and species were 140(21.80%), 87(13.55%), 71(11.06%) and 72(11.21%) isolates respectively (Table 3).

Table 4: Antibiotic sensitivity pattern of Gram positive cocci (n=232)

Antibiotics	*Staphylococcus aureus(193)*	*Enterococcus (22)*	*CONS(17)*
Cotrimazazole	82(42.48%)	15 (68.18%)	7 (41%)
Erythromycin	149(77.20%)	18(81.81%)	5(29.40%)
Clindamycin	162(83.90%)	18(81.81%)	6(35.20%)
Chloramphenicol	131(67.80%)	20(90.90%)	6(35.20%)
Tetracycline	104(53.80%)	19(86.36%)	8(47.05%)
Linezolid	193(100%)	22(100%)	17(100%)
Vancomycin	193(100%)	22(100%)	17(100%)
Gentamicin	171(88.60%)	16(72.72%)	7(41%)
Ciprofloxacin	102(52.80%)	22(100%)	6(35.29%)

All 193(100%) isolated *Staphylococcus aureus* were sensitive to Vancomycin and linezolid.

Table 5: Antibiotic sensitivity pattern of MSSA and MRSA

Antibiotics	*MSSA(n=116)*	*MRSA(n=77)*
Cotrimazazole	47(40.51%)	35(45.45%)
Erythromycin	114(98.27%)	35(45.45%)
Clindamycin	116(100%)	46(59.74%)
Chloramphenicol	95(81.89%)	36(46.75%)
Tetracycline	65(56.03%)	39(50.65%)
Linezolid	116(100%)	77(100%)
Vancomycin	116(100%)	77(100%)
Gentamicin	103(88.79%)	68(88.31%)
Ciprofloxacin	66(56.89%)	36(46.5%)

Among the 193(30.06%) *Staphylococcus aureus* 77(39.90%) were MRSA.

Table 6: Antibiotic sensitivity pattern of Gram negative bacilli

Antibiotic	*Pseudomonas aeruginosa (n=87)*	*Escherichia coli (n=71)*	*Proteus mirabilis (n=40)*	*Acinetobacter baumanii (n=72)*
Ampicillin	4(4.60%)	9(12.68%)	16(40%)	47(65.28%)
Amoxyclavulanic acid	40(45.98%)	16(22.54%)	28(70%)	40(55.56%)
Gentamicin	22(25.29%)	42(59.15%)	22(55%)	53(77.78%)
Amikacin	45(51.72%)	52(73.23%)	40(100%)	49(68.06%)
Ciprofloxacin	32(36.78%)	11(15,49%)	22(55%)	58(80.86%)
Ceftazidime	7(8.04%)	7(9.86%)	19(47.50%)	25(34.72%)
Cefotaxime	57(65.52%)	30(42.25%)	19(47.50%)	43(59.72%)
Meropenem	55(63.22%)	56(78.87%)	40(100%)	67(93.06%)
Piperacillin –tazobactam	52(59.77%)	66(92.95%)	40(100%)	72(100%)

Highest rate of susceptibility was seen toward Piperacillin-tazobactam(87.75%), followed by Meropenem (83.40%).

Table 7: Antibiotic sensitivity pattern of *Klebsiella pneumoniae*

Antibiotic	*Klebsiella pnuemoniae (n=140)*
Ampicillin	16(11.43%)
Amoxyclavulanic acid	24(17.14%)
Gentamicin	90(64.28%)
Amikacin	123(87.86%)
Ciprofloxacin	49(35%)
Ceftazidime	32(22.86%)
Cefotaxime	114(81.43%)
Meropenem	131(93.57%)
Piperacillin –tazobactam	112(80%)

Among 140 *Klebsiella* isolated in our study 87.86% were sensitive to amikacin and 80% were sensitive to Piperacillin-Tazobactam as shown in Table 7.

Table 8: Total number of ESBL

Organism	Number (n=410)	Percentage (%)
ESBL producer	237	57.80%
Non ESBL producer	173	42.20%

Total ESBL producing organisms were 237(57.80%).

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 pneumoniae, Proteus mirabilis*, and Gram positive cocci such as *Staphylococcus aureus* and *Enterococci* are the common causative agents. In this study, both gram positive and gram negative pathogens were isolated from samples. The predominant pathogens were gram negative bacteria (63.86%). It was agreed with studies done by Swati Duggal et al. [9] and Shama et al. [10] which showed dominant pathogens as Gram negative bacteria. The most common gram negative being *Klebsiella pneumonia* 140(21.81%), followed by *Pseudomonas aeruginosa* 87(12.61%), *Acinetobacter baumanii* 72(11.21%), *E coli* 71(11.06%) and *Proteus mirabilis* 40(6.23%) . These organisms are commonly found in hospital environment. They tend to be resistant to common antiseptics and are also multidrug resistant.

Among gram positive pathogens, *Staphylococcus aureus* was commonly isolated followed by *Enterococci* and CONS which correlates with the study done by Kumari PH et al. [11] *Staphylococcus aureus* being normal flora of the skin, is usually associated with pyogenic infections. [12]

ß-lactamases, which are responsible for resistance of ßlactam group of antibiotics, hydrolyse the amide bond of the four-membered characteristic ß-lactam ring, thus rendering the antimicrobial ineffective. [13] Present study showed ESBL positivity in 237 (57.80%) Gram negative isolates.

Methicillin-resistant Staphylococcus aureus (MRSA) strains are resistant to a large group of antibiotics called beta-lactams, including

penicillins 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 β-lactam antibiotics. [14,15] In present study, MRSA was detected in 77(39.90%) *Staphylococcus aureus* isolates.

Most of Gram negative isolates were susceptible to piperacillin / tazobactum (87.75%) and meropenem (83.40%) which is similar to study done by Rameshkannan S. et al. which showed maximum susceptibility to these antibiotics. [16] Most of Gram positive isolates were sensitive to vancomycin 193(100%) and linezolid 193(100%) which is same as the results of studies conducted by Verma P[17] and Shittu AO et al. [18] 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, Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa* isolates from pus samples. Hence formulation of antibiotic policies and infection control measures suitable has to be considered essential. [19]

CONCLUSION

The spread of beta-lactamase producing organisms has been increasing. Present study showed increased resistance to beta-lactam antibiotics which is a serious problem. To combat resistance irrational use of antibiotics should be avoided. Also regular surveillance helps in implementing better therapeutic strategies to reduce morbidity and mortality.

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