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Bacteriological profile of pus samples and their antibiotic susceptibility pattern

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ABSTRACT

Introduction: 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.

Materials and Methods: A retrospective study was carried out from January to June 2019. 108 pus samples collected during study period were included. The samples were cultured on Blood and MacConkey agar. After aerobic incubation at 37^{o}C for 18-24 hrs, organisms were identified by standard methods and antibiotic susceptibility was tested by Kirby Bauer disc diffusion method. ESBL was detected by combined disk test and Methicillin resistant Staphylococcus aureus (MRSA) by Cefoxitin 30 μg disc.

Results: Of 118 pus samples collected, 101(85.5%) were positive cultures and no growth was in 17(14.4%) samples. S. aureus 27(22.9%) was most common Gram positive isolate and Pseudomonas spp. 17(14.4%), was most common gram negative isolate. ESBL positivity was seen in 38(61.2%) and MRSA in 13(48.1%) S. aureus isolates. Most of gram negative isolates were susceptible to piperacillin / tazobactum 55(88.7%) and meropenem 53(85.4%) and gram positive isolates to vancomycin 27(100%) and linezolid 25(92.5%).

Conclusion: The spread of beta-lactamase producing organisms has been increasing. Our 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.

1. Introduction

Pyogenic infections refer to infection that causes pus formation and are characterized by several local inflammations, usually multiplication of microorganism.[1] 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.[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]

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

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of particular antibiotics.[9] The routine use of antibiotics has resulted in wide spread antibiotic resistance especially within the gram negative organisms.[10] Bacteria have the ability to acquire resistance and can transfer the resistance from one bacteria to another.[11] Earlier, such multidrug resistant organisms were common in immunosuppressed patients but now, reports are showing such infections in normal healthy individuals. Also, such drug-resistant infections may complicate the newly emerging infectious diseases.[12] The emergence of high anti-microbial resistance among bacterial pathogens has made the management and treatment difficult.[13] It is ideal to give proper antibiotic after culture and sensitivity of the wound swab or pus.[14] The present study aimed to detect common bacteriological profile and their antibiotic susceptibility profile from wound infection.

2. Materials and Methods

A retrospective study was conducted at Chamarajanagar Institute of medical sciences, Chamarajanagar from January to June 2019. All pus samples collected during the study period 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 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 $\geq$ 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 $\leq$ 21 mm was considered as methicillin resistant.

2.1. Statistical analysis

Analysis was done using MS Excel.

2.2. Ethical considerations

Ethical clearance was obtained from the Institutional Ethical clearance committee of Chamarajanagar Institute of medical sciences, Chamarajanagar.

3. Results

Of 118 pus samples collected, 101(85.5%) were positive cultures, which included 62 (52.5%) Gram negative and 39(33.1%) Gram positive bacteria. Mixed growth was seen in 5(4.2%) samples and no growth in 17(14.4%) samples. Middle age group of 19-45 years 51 (43.2%) was most commonly affected age group. Males 75(63.6%) were commonly affected than females 43(36.4%). S. aureus 27(22.9%) was most common isolate followed by Pseudomonas spp. 17(14.4%), E. coli 16(13.6%), CONS 10(8.5%), Citrobacter spp. 9(7.6%), Klebsiella pneumoniae and Enterobacter spp. 6(05.1%) each, Proteus spp. 5(04.2%), Acinetobacter spp. 3(02.5%) and Enterococcus spp. 2(1.7%). ESBL positivity was seen in 38(61.2%) Gram negative isolates and most were susceptible to piperacillin / tazobactum 55(88.7%) and meropenem 53(85.4%). MRSA was detected in 13(48.1%) S. aureus isolates and were susceptible vancomycin 27(100%) and linezolid 25(92.5%).

4. 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.[16]

In this study, both gram positive and gram negative pathogens were isolated from samples. The predominant pathogens were gram negative bacteria (52.5%). It was agreed with studies done by Swati Duggal et al.[17] and Shama et al.[18] which showed dominance pathogens as Gram negative bacteria. The most common gram negative being Pseudomonas spp. 17(14.4%), followed by E. coli 16(13.6%), Citrobacter spp. 9(7.6%), Klebsiella pneumoniae and Enterobacter spp. 6(05.1%) each, Proteus spp. 5(04.2%) and Acinetobacter spp. 3(02.5%). 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 CONS and Enterococci which correlates with the study

Table 1: Age and gender wise distribution

Age (years)	No. of isolates No. (%)	Gender	
		M No. (%)	F NO. (%)
1 – 18	27 (22.9)	17 (14.4)	10 (08.5)
19 -45	51 (43.2)	31 (26.3)	20 (16.9)
> 45	40 (33.9)	27 (22.9)	13 (11.0)
Total	**118 (100)**	**75 (63.6)**	**43 (36.4)**

Table 2: Organisms isolated from pus culture

Organisms	No. of organisms	Percentage (%)
Staphylococcus aureus	27	22.9
CONS	10	08.5
Enterococcus spp.	02	01.7
Escherichia coli	16	13.6
Klebsiella pneumoniae	06	05.1
Proteus spp.	05	04.2
Citrobacter spp.	09	07.6
Acinetobacter spp.	03	02.5
Enterobacter spp.	06	05.1
Pseudomonas spp.	17	14.4
No growth	17	14.4
Total	**118**	**100**

Table 3: Antibiotic susceptibilitypattern of Gram negative isolates

Organisms	AMP	AMC	G	AK	CIP	CAZ	CTX	MRP	PIT
Escherichia coli (n=16)	06 (37.5)	10 (62.5)	08 (50.0)	10 (62.5)	12 (75.0)	07 (43.7)	07 (43.7)	12 (75.0)	13 (81.2)
Klebsiella spp. (n=06)	02 (33.3)	03 (50.0)	05 (83.3)	04 (66.6)	04 (66.6)	03 (50.0)	03 (50.0)	05 (83.3)	04 (66.6)
Proteus spp. (n=05)	04 (80.0)	05 (100)	04 (80.0)	04 (80.0)	05 (100)	04 (80.0)	04 (80.0)	05 (100)	05 (100)
Citrobacter spp. (n=09)	05 (55.5)	05 (55.5)	06 (66.6)	06 (66.6)	08 (88.8)	05 (55.5)	05 (55.5)	09 (100)	09 (100)
Acinetobacter spp. (n=03)	02 (66.6)	02 (66.6)	02 (66.6)	02 (66.6)	02 (66.6)	02 (66.6)	02 (66.6)	01 (33.3)	02 (66.6)
Enterobacter spp. (n=06)	03 (50.00)	03 (50.00)	04 (66.6)	05 (83.3)	04 (66.6)	03 (50.00)	03 (50.00)	06 (100)	05 (83.3)
Pseudomonas spp. (n=17)	05 (29.4)	11 (64.7)	11 (64.7)	13 (76.5)	09 (52.9)	09 (52.9)	09 (52.9)	15 (88.2)	16 (94.1)
Total (n=62)	**27 (43.5)**	**39 (62.9)**	**40 (64.5)**	**44 (70.9)**	**44 (70.9)**	**33 (53.2)**	**33 (53.2)**	**53 (85.4)**	**55 (88.7)**

AMP – Ampicillin, AMC – Amoxyclav, G – Gentamycin, AK – Amikacin, CIP – Ciprofloxacin, CAZ – Ceftazidime, CTX – Cefotaxime, MRP – Meropenem, PIT – Piperacillin-Tazobactum

done by Kumari PH et al.[19] Staphylococcus aureus being normal flora of the skin, is usually associated with pyogenic infections.[20]

ß-lactamases, which are responsible for resistance of ß-lactam group of antibiotics, hydrolyse the amide bond of the four-membered characteristic ß-lactam ring, thus rendering the antimicrobial ineffective.[21] Present study showed ESBL positivity in 38(61.2%) 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.[22,23] In present study, MRSA was detected in 13(48.1%) S. aureus isolates. Most of Gram negative isolates were susceptible to piperacillin / tazobactum 55(88.7%) and meropenem 53(85.4%) which is similar to study done by Rameshkannan S. et al. which showed maximum susceptibility to these antibiotics.[24] Most of Gram positive isolates were sensitive to vancomycin 27(100%) and linezolid 25(92.5%) which is same as the

Table 4: Antibiotic susceptibility pattern of Gram positive isolates

Organisms	AMP No. (%)	G No. (%)	AK No. (%)	CIP No. (%)	COT No. (%)	E No. (%)	CD No. (%)	C No. (%)	TE No. (%)	LZ No. (%)	VA No. (%)
Staphylococcus aureus (n=27)	00 (00)	05 (38.5)	07 (53.8)	03 (23.1)	04 (30.7)	08 (61.5)	06 (46.1)	07 (53.8)	08 (61.5)	12 (92.3)	13 (100)
MRSA 13 MSSA 14	05 (35.7)	11 (78.5)	12 (85.7)	07 (50.0)	12 (85.7)	07 (50.0)	08 (57.1)	12 (85.7)	12 (85.7)	13 (92.8)	14 (100)
Total (n=27)	**05 (18.5)**	**16 (59.2)**	**19 (70.3)**	**10 (37.0)**	**16 (59.2)**	**15 (55.5)**	**14 (51.8)**	**19 (70.3)**	**20 (74.1)**	**25 (92.5)**	**27 (100)**
CONS (n=10)	00 (00)	06 (60.0)	08 (80.0)	04 (40.0)	04 (40.0)	03 (30.0)	06 (60.0)	06 (60.0)	05 (50.0)	09 (90.0)	10 (100)
Enterococcus (n=02)	01 (50.0)	01 (50.0)	-	01 (50.0)	-	-	-	-	01 (50.0)	02 (100)	02 (100)

AMP – Ampicillin, G – Gentamycin, AK – Amikacin, CIP – Ciprofloxacin, COT- Cotrimoxozole, E – Erythromycin, CD – Clindamycin, C- Chloramphenicol, TE- Tetracycline, LZ- Linezolid, VA- Vancomycin

Table 5: Number of ESBL producers

No. of Gram negative isolates	ESBL producers No. (%)
62	38(61.2)

results of studies conducted by Verma P[25] and Shittu AO et al.[26]

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 suitable has to be considered essential.[27]

5. 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.

6. Source of Funding

None.

7. Conflict of Interest

None.

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