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Original Research Article

Study of bacteriological profile and antibiotic susceptibility pattern of pus isolates in tertiary care hospital

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ABSTRACT

Background: Surgical wound causes invasion of pathogens causing surgical site infections which are commonly polymicrobial in nature. This pus forming infection causes delayed wound healing, wound dehiscence and wound breakdown contributing to important healthcare associated infections (HAI) Multidrug resistance has emerged among organisms isolated in pus sample due to failure of appropriate use of antibiotics.

Objectives of the study: 1. To study the bacteriological profile of pus samples 2. To determine the antibiotic susceptibility pattern of isolated pathogens from pus samples.

Materials and Methods: The study was conducted in department of microbiology, Shimoga institute of medical sciences, Shimoga from January 2018 to June 2018.All pus samples were processed on blood agar, MacConkey agar and incubated at 37°c under aerobic conditions for 24 hours. The organisms were identified as per standard conventional methods. The antimicrobial susceptibility tests were done by Kirby–Bauer's Disk Diffusion method on Mueller–Hinton Agar and interpreted as per clinical laboratory standard institution guidelines (CLSI).

Result: Out of 350 samples 250 were culture positive 100 were culture negative. Among culture positive most common organism isolated was followed by *Staphylococcus aureus* 86(34.45%), *Klebsiella spp* 72(28.8%), *Pseudomonas Aeruginosa* 55(22%), *E. coli* 20(8%), *CoNS* 9(3.6%), *Proteus spp* 2(0.8%), *Enterococcus spp* 3(1.2%) *Acinetobacter spp* 3(1.2%), *Citrobacter spp* 3(1.2%). Gram positive organisms were most sensitive for linezolid, vancomycin and least sensitive to cefoxitin, erythromycin. Gram Negative Organisms Were Most Sensitive for Imipenam, Piperacillin tazobactam and least Sensitive for Ampicillin-sulbactam, Ciprofloxacin

Conclusion: *Staphylococcus aures* is most common etiology of pus forming infection most importantly surgical site infections (SSI). MRSA prevalence in hospital set up indicates the failure of proper infection control practices implementation in the hospitals causing healthcare associated infections (HAI). Emergence of multidrug resistance among the pus isolates is because of non-judicious use of antibiotics.

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1. Introduction

Wound causes exposure of underlying subcutaneous tissue to pathogenic microorganisms which can be either monomicrobial or polymicrobial. Wound can be clean, clean contaminated, contaminated, dirty This causes pus formation which is yellow, white colour which consists of WBC's which are dead, cellular debris, necrotic tissues. Pus can be PATOS (present at time of surgery) or pus can be seen in patient who had surgery within past 30 days or 90 days with implants. Surgical site infections(post-operative wound infections) leading to

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nosocomial infections and skin and soft tissue infections (SSTIs) due to trauma, burn injuries contribute to morbidity and mortality of the patient.[1,2] Immunocompromised conditions like diabetes mellitus, patient on corticosteroids, chemotherapy undergoing patients where invasion and survival of pathogens causes wound infection and delayed wound healing. pus appearance in the post-operative wound itself contribute as important factor of infection.[2,3] These may be endogenous or exogenous or it may be polymicrobial or monomicrobial in nature. Both aerobic and anaerobic bacteria have been found in hospital acquired infections, especially postoperative wound infections. *Pseudmonas aeruginosa* is the most common organism isolated in burn patients.[3] Most common organisms causing pyogenic infection includes gram positive cocci such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus spp* and gram negative bacilli such as *Pseudomonas aeruginosa*, *Klebsiella spp*, *E. coli*.[2,3] Healthcare associated infections (HAI) prevention of SSI is by implementation of infection control practices in the hospitals. Antibiotic sensitivity pattern of microorganisms isolated from pus sample vary from one geographical area to another geographical area. Therefore the choice of antibiotic for empirical therapy should be based on the local antibiotic susceptibility pattern. Various strategies must be implemented for rationalizing the use of antibiotics in hospital routinely and minimize the emergence of multidrug resistant bacteria among the pus isolates and prevent the risk of global public health problem.[2,3]

2. Materials and Methods

A cross-sectional prospective study was conducted at Department of microbiology, Shimoga institute of medical sciences, Shimoga from January 2018 to June 2018 for duration of 6 months. All pus samples of inpatient department (IPD) and outpatient department (OPD) were included in the study.

Pus sample collection: Under aseptic precautions the pus specimens were collected in sterile swabs or by aspiration of pus in sterile syringes and sample transported to the microbiology laboratory. Two sterile cotton swabs of pus sample collected for each patient. One sterile cotton swab was used to perform gram stain smear another pus swab was sent for culture. All pus samples were processed on MacConkey agar, blood agar, chocolate agar and incubated at 37°c under aerobic conditions for 24 hours. Identification of the bacterial colony followed by gram stain smear and subjecting the culture growth for, biochemical reactions as per standard conventional microbiological methods.[4,5] The antimicrobial susceptibility tests were done by Kirby–Bauer's Disk Diffusion method on Mueller–Hinton Agar using bacterial suspensions matched with McFarland Standard and interpreted as per clinical laboratory standard institution guidelines (CLSI).[5,6]

2.1. Antimicrobial susceptibility testing

Antibiotic susceptibility testing was carried out by Kirby Bauer Disk Diffusion method. Antibiotics discs used are Ampicillin (Amp)-10μg, amoxyclav (20/10μg), gentamicin (10μg), amikacin (30μg), ciprofloxacin (5μg), Levofloxacin (5μg) ceftazidime (30μg), Cefotaxime (30μg), Ampicillin sulbactam (20μg), Aztreonam (10μg) Imipenem (10μg) and Piperacillin – Tazobactum (100/10μg). Azithromycin (30μg), Cefoxitin ((30μg), Doxycycline (30μg) Cotrimoxazole (1.25μg /23.75μg), Erythromycin (5μg), Clindamycin (2μg), Linezolid (30μg), Vancomycin (30μg).[6]

3. Result

The present study was conducted at Department of microbiology, Shimoga institute of medical sciences, Shimoga from January 2018 to June 2018. Out of 350 samples 250 were culture positive 100 were culture negative. Most common organism isolated was followed by *Staphylococcus aureus* 86(34.45%), *Klebsiella spp* 72(28.8%), *Pseudomonas Aeruginosa* 55(22%), *E.Coli* 20(8%), *CoNS* 9(3.6%), *Proteus spp* 2(0.8%), *Enterococcus spp* 3(1.2%) *Acinetobacter spp* 3 (1.2%), *Citrobacter spp* 3(1.2%).

Among gram negative organisms *Klebsiella spp* was highly sensitive to Aztreonam (88%), Amikacin (78%), Gentamicin (64.6%) and least sensitive to Ciprofloxacin (9.4%), Levofloxacin (9.4%), Piperacillin-tazobactam (10.4%). *Pseudomonas aeruginosa* was highly sensitive to Piperacillin-Tazobactam (86.6%), Amikacin (75.8%), Imipenem (66.4%) and least sensitive to Ampicillin-sulbactam (6.2%), Amoxicillin-clavulanic acid (10.2%), Levofloxacin (18.5%). Among gram positive organisms *Staphylococcus aureus* was highly sensitive to Linezolid (100%), Vancomycin (100%), Doxycycline (68.4%), Cotrimoxazole (66.6%) and least sensitive to Cefoxitin ((28.6%)), Erythromycin (10.5%), Azithromycin (15.5%). *Coagulase negative staphylococcus* (*CoNS*) was highly sensitive to Linezolid (100%), Vancomycin (100%), Cotrimoxazole (68%) least sensitive to Erythromycin (18%), Azithromycin (25.5%). Cefoxitin ((32%)

4. Discussion

According to our study most common organism isolated was *Staphylococcus aureus* 86(34.45%) followed by, *Klebsiella spp* 72(28.8%), *Pseudomonas aeruginosa* 55(22%), *E. coli* 20(8%), *CoNS* 9(3.6%). Similar study done by Jamatia et al shows *Staphylococcus aureus* (30.11%) followed by *Pseudomonas aeruginosa* (21.02%), *Klebsiella spp*. (17.03%), *E. coli* (13.63%), *Proteus spp*. (6.25%).[7] Similar study done by murugesan et al.,(2017) bacteriological profile shows *Staphylococcus aureus* (66.41%), *Klebsiella species* (22.13%), *Pseudomonas*

Table 1: Antibiotic sensitivity pattern of gram negative organisms isolated from pus specimens

	Klebsiella spp (%)	*Pseudomonas* spp (%)	*E. coli* (%)	*Proteus spp* (%)	*Acinetobacter spp* (%)
Imipenem	-	66.4	88.4	100	100
Piperacillin-tazobactam	10.4	86.6	32.6	100	100
Gentamicin	64.6	10.8	78.8	100	100
Ampicillin sulbactam	-	6.2	22.7	0	33.33
Aztreonam	88	44.8	45.5	66.6	100
Ciprofloxacin	9.4	36.6	31.6	66.6	33.33
Levofloacin	9.4	18.5	16.4	0	0
Amikacin	78	75.8	66.3	100	100
Cotrimoxazole	48.4	25.5	74.3	100	100
Cefotaxim	46	43.6	49.8	100	100
Amoxyclavulanic acid	4.7	10.2	22.7	33.3	33.33
Ceftazidime	23.4	26.7	35.5	66.66	100

Table 2:

	S. aureus	*CONS*	*Enterococcus spp*
Linezolid	100	100	100
Vancomycin	100	100	100
Clindamycin	52.4	66	-
Erythromycin	10.5	18	-
Azithromycin	15.5	25.5	-
Cefoxitin	28.6	32	-
Cotrimoxazole	66.6	68	-
Doxycycline	68.4	58.3	-
Gentamycin	63.4	60.6	0
Ampicillin	10.45	11	0

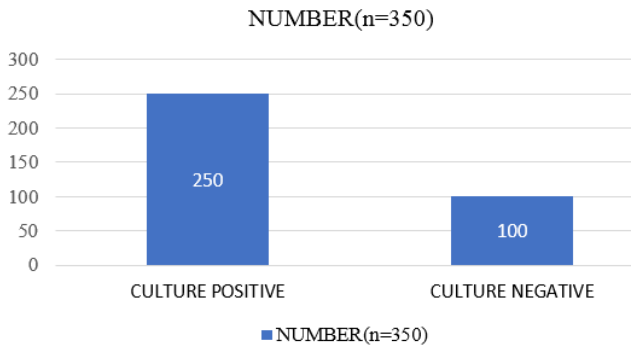

Figure 1: Culture positive and Culture negative pus samples

aeruginosa (11.15%)[8] Study done by Kumar et al.,(2017) shows *Staphylococcus aureus* (47.55%), *Pseudomonas aeruginosa* (n=101, 35.31%), *Klebsiella spp* (n=35, 12.23%) and *E. coli* (n=14, 4.89%)[9] Most commonest causative agent of skin and soft tissue infections is *Staphylococcus aureus*. According to our study *Staphylococcus aureus* was highly sensitive to Linezolid (100%), Vancomycin (100%) and least sensitive to Cefoxitin ((28.6%)), Erythromycin (10.5%), Azithromycin (15.5%). Similar study done by Murugesan et al showed *Staphylococcus aureus* highly sensitivetio Vancomycin (94.25%), Cloxacillin (70.11%) and resistance towards Erythromycin (55%) and Methicillin (25%).[8] Similar study done by Kumar et al.,(2017) shows high sensitivity to linezolid (91.17%) Vancomycin (79.41%) and methicillin resistance (38.23%).[9] Similar study done by Mohanty et al shows methicillin resistance 38.56% and study by Singh et al shows methicillin resistance 45%.[10,11] Similar study done by Rai et el.,(2012) shows high sensitivity for Cefoxitin (81%), Gentamicin (76%), Erythromycin (72%) when compared to our study.[12] Study by Khanam et al shows high antibiotic resistance of *Staphylococcus aureus* to penicillin (84.5% to penicillin and 63.6% to ampicillin)[13] Methicillin resistant *staphylococcus aureus* (MRSA) is important hospital acquired infection (HAI) and MRSA outbreak contributes to mortality and morbidity. In our study *Klebsiella spp* was highly sensitive to Aztreonam (88%), Amikacin (78%), Gentamicin (64.6%) and least sensitive to Ciprofloxacin (9.4%), Levofloxacin(9.4%), Piperacillin-tazobactam (10.4%). Study by Gomatheswari et al shows high sensitivity to Imipenem (83%) followed by Amikacin (68%) and high resistance to Ampicillin(83%), Ceftazidime-clavulanic acid (58%).[14] Similar study done by Jamatia et al shows *Klebsiella spp* highest resistance to Penicillin (100%), Amoxicillin (100%), Amoxiclavulanic acid(100%).[7] In

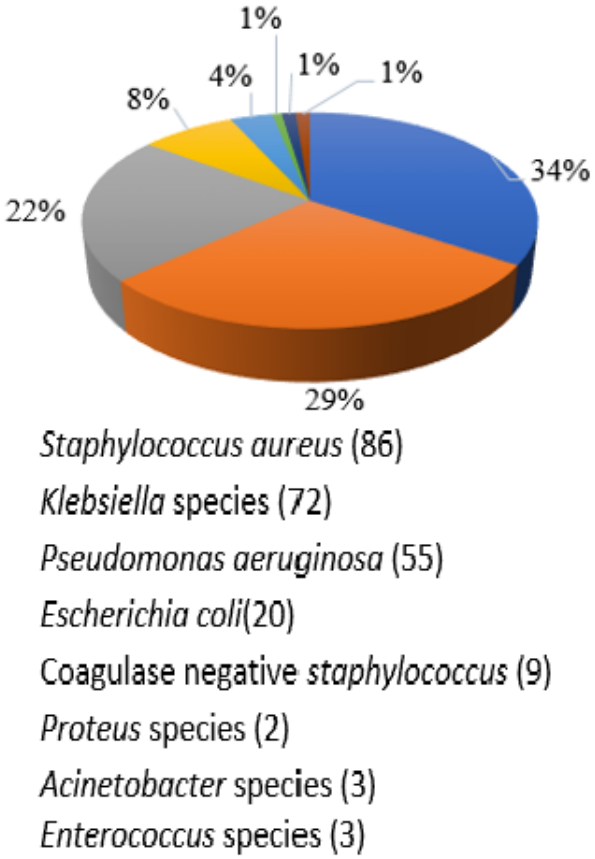

Figure 2: Bacteriological profile of pus samples

our study *Pseudomonas aeruginosa* was highly sensitive to Piperacillin-Tazobactam (86.6%), Amikacin (75.8%), Imipenem (66.4%) and least sensitive to Ampicillin-sulbactam (6.2%), Amoxicillin-clavulanic acid (10.2%), Levofloxacin (18.5%). Similar study done by Kumar et al high sensitivity was seen in Amikacin (90%), Gentamicin (88%) Levofloxacin (71%), Ciprofloxacin (62%) and least sensitive to Cefotaxime (92%), Cloxacillin (90%), Ampicillin (58%).[9] Similar study by Kanam et al showed *Pseudomonas aeruginosa* (14%) as commonest organism isolated and highest rate of susceptibility was seen toward cefuroxime (87.5%), followed by Cefaridine (62.5%). Study by Rai et al shows Highest rate of susceptibility was toward ciprofloxacin (51%), followed by tobramycin (44%) *Pseudomonas aeruginosa* is opportunistic nosocomial pathogen and commonest organism isolated froms burns patients. According to our study *Coagulase negative staphylococcus* (*CoNS*) was highly sensitive to Linezolid (100%), Vancomycin (100%), Cotrimoxazole (68%) least sensitive to Erythromycin (18%), Azithromycin (25.5%). Cefoxitin ((32%). Similar study done by Bora et al shows isolation of *Staphylococcus epidermidis* (46.6%), Staphylococcus hemolyticus 42.3%. *Staphylococcus epidermidis* shows high degree of resistance to penicillin (97%), oxacillin (64.7%) and high sensitivity to Linezolid (100%), Vancomycin (100%).[15] *MRCoNS* is important agent of hospital acquired infection which has potential to transfer resistance mechanism to *Staphylococcus aureus* present on skin and hospital settings.[15,16]

5. Conclusion

Majority of pus samples showed the isolation of more than one microorganism (polymicrobial) which results delay in wound healing. *Staphylococcus aures* is the most common organism isolated from pus samples and is important etiology of healthcare associated infections (HAI) mainly surgical site infections (SSI). Infection control practices like pre-operative screening for carriers, Decoloniztion with mupirocin ointment for *Staphylococcus aureus* carriers undergoing surgeries, surgical antimicrobial prophylaxis 120min prior to the surgery, Peri-operatively surgical site skin preparation, hand scrub before and in between cases, post operatively surgical dressing, hand hygiene to be followed to prevent the delayed wound healing and also to prevent healthcare associated infections. Most of the organisms showed multiple antibiotic resistance due to extended spectrum beta lactamases. Strategies for rationale use of antibiotics to be followed to prevent the emergence of multidrug resistant bacteria among the pus isolates and to reduce morbidity and mortality.

6. Source of Funding

None.

7. Conflict of Interest

None.

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