Microbial Profile and Antibiogram of Pus Isolate in a Tertiary Care Hospital of Western Odisha

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ABSTRACT

BACKGROUND

Pus sample is one of the major samples received in Microbiology laboratory. Overall incidence of wound sepsis in India is 10-33%. A predictable microbial profile in wound infection is very much important for clinician to start empirical therapy and also to combat drug resistance. We wanted to assess the microbial profile and antibiogram in pus isolates causing wound infection in a tertiary care centre of Western Odisha.

METHODS

This is a cross-sectional descriptive study. 287 pus samples in total received from different departments of Hitech Medical College, Rourkela, from June 2019 to Dec. 2019 were processed using standard microbial culture methods and antibiotic sensitivity was done as per CLSI guideline.

RESULTS

Among 287 samples, 187 (65%) showed positive growth. Among 187 positive samples, 64% were from male population. Middle age group (21-40 and 41-60) was most affected age group (36% and 37.4% respectively). Most commonly isolated bacteria were *Staphylococcus aureus* (38%) followed by *Pseudomonas* species (17.10%). Least common bacteria were *Streptococcus pyogenes* (0.5%). Most of the gram-negative bacilli showed high sensitivity towards imipenem (53-93%), meropenem (80-100%), piperacillin tazobactam (67-100%) and amikacin (67-88%) but lower sensitivity to 3rd generation cephalosporins (36-73%), cotrimoxazole (60-80%), amoxiclav (20-75%) and fluoroquinolones (42-73%). *Staphylococcus aureus* showed high sensitivity to linezolid (92%), aminoglycoside (84%), clindamycin (88%) and cotrimoxazole (72%) but lower sensitivity to erythromycin (25%), amoxiclav (40%) and fluoroquinolones (44%).

CONCLUSIONS

Staphylococcus aureus and *Pseudomonas* species were the most common bacteria isolated in our study with variable antibiogram. Pus aspirate was better sample than pus swab. A continuous inspection should be carried out to monitor the antibiogram of wound isolate to formulate and implement antibiotic policy in our hospital.

KEY WORDS

Pus, Profile, Bacteria, Antibiogram

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DOI: 10.14260/jemds/2020/289

Financial or Other Competing Interests: None.

How to Cite This Article:
Mukherjee S, Mishra S, Tiwary S. Microbial profile and antibiogram of pus isolate in a tertiary care hospital of Western Odisha. J. Evolution Med. Dent. Sci. 2020;9(16): 1325-1330, DOI: 10.14260/jemds/2020/289

Submission 18-02-2020,
Peer Review 04-04-2020,
Acceptance 10-04-2020,
Published 20-04-2020.

BACKGROUND

Infection of wound is of two types depending on the causative organism- pyogenic and non-pyogenic. If the bacterium is pyogenic (pus forming), it produce pyogenic infection which is characterized by local and systemic inflammation. Non-pyogenic wound infection is usually caused by atypical mycobacteria, fungus and virus Inflammation at local sites invites leukocytes to kill the bacteria. When these leukocytes are dead they form white to yellow colour fluid that is called pus.[1,2] Surgical intervention, burn, bites, abrasion, minor cut, laceration, crush injury or gunshot injury result in loss of intact skin and produce wound.[3] Presence of moisture, warmth, nutrition in wound attract bacteria from skin surface, environment or from patients own flora and these bacteria proliferates and liberates different virulence factor and produce wound infection[4]. If the wound is due to surgical incision it is called surgical site infection.[5] Surgical site infection (SSI) develops within 30 days after a surgical procedure or within 1 year if an implant is placed. SSI is of three types, depending on the depth of infection: superficial, incisional/deep incisional, and organ/space.[5] After end of the surgical procedure surgical site is colonised by bacteria and if the bacteria is virulent and host defence is weak then colonised bacteria produce infection. SSI contributes to approximately 20% of hospital acquired infection. Surveillance data suggest that etiological profile of SSI is almost same for last 10 to 15 years although proportion of them has been changed with increased trend of drug resistant bacteria.[5] Post-operative wound infection is the most common wound infection and considered as having a polymicrobial aetiology, involving both aerobic and anaerobic microorganisms. Intra-abdominal infections usually caused by bacterial flora of the resected organ. Although aerobic and anaerobic both type of bacteria produce wound infection most of the cases occur due to aerobic bacteria. Example of aerobic bacteria are Gram positive cocci like *Staphylococcus aureus*, *Enterococci* and Gram negative bacteria like *E. coli*, *Klebsiella* pneumoniae, *Pseudomonas aeruginosa*, *Acinetobacter*, *Proteus* species etc.[6] Besides bacteria, virus, protozoa, fungus can also cause wound infection and sometimes they may coexist with one or more bacteria in a single wound[7]. In different study worldwide etiological profile of wound infection is almost same with slight variation but their antibiogram varies in different geographical area. 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.[8] 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 Western Odisha we have conducted a research to find out microbial profile and antibiogram of wound isolates in tertiary care hospital of Western Odisha.

METHODS

This is a descriptive study conducted in the Department of Microbiology, Hitech Medical College, Rourkela, Western Odisha, over a period of 6 months (august 2019 to Jan. 2020). A total of 287 samples were collected by sterile syringe aspiration, sterile swab and tissue scraping. from Indoor and Outdoor of different departments like Surgery, Orthopaedics, Medicine, ENT, OBG etc. at Hitech Medical College, Rourkela.

Sample Processing

Cotton swabs placed in screw capped tubes, pus sample in sterile disposable syringe and tissue scraping in sterile container were immediately sent to Microbiology Laboratory. All samples were inoculated into Blood agar, MacConkey agar, and Nutrient agar. Brain heart infusion broth (BHIB) media was inoculated as back up. All the media were incubated for 24 hrs. at 37^0C. Next day growth was observed, and identification was done using standard protocol using Gram stain, motility test, Biochemical test like Catalase, Coagulase, Indole, MR, VP, Citrate, urease, PPA, oxidase test etc. If no growth was found after 24 hrs., subculture was done from BHIB and incubated for 24 hrs. No growth was declared only after incubating media for 48 hrs. Antibiotic sensitivity testing of bacterial isolates was performed by Modified Kirby Bauer disc diffusion method on Mueller Hinton agar media by antibiotics recommended by CLSI guidelines.[9]

Antibiotic discs used for Gram negative bacilli were Amikacin (30µ), Cefoperazone (75µ), Cefoperazone-sulbactam (75/30µ) Piperacillin-tazobactam, (100/10µ) Piperacillin (100µ), Cotrimoxazole, Netilmicin (30µ) Tigecycline, Amoxiclav (20/10µ) Ciprofloxacin (5µ), Ceftriaxone (30µ) Gentamycin (10µ), Meropenem (10µ), Imipenem (10µ) and special antibiotic for *Pseudomonas* were Ceftazidime (30µ) Ceftazidime- clavulanic acid (30/10 µ), Azithromycin (15µ). Linezolid (30µ), Ciprofloxacin (5µ) Cotrimoxazole, Gentamicin (10µ) Erythromycin (15µ), Clindamycin (2µ), Tigecycline, were put for any catalase positive gram-positive cocci growth (*Staphylococcus*). Ampicillin (10µ), Ampicillin sulbactam (10/10µ), Teicoplanin, Linezolid (30µ), Nitrofurantoin (100µ) Ciprofloxacin (5µ), Cotrimoxazole and Gentamycin (10µ) were put for any catalase negative gram-positive cocci growth (*Enterococci*).

Quality Control

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.

Data analysis was performed using SPSS version 17.

RESULTS

Among 287 samples 187 samples (65%) showed positive growth for single bacteria and 100 samples (35%) showed no growth.

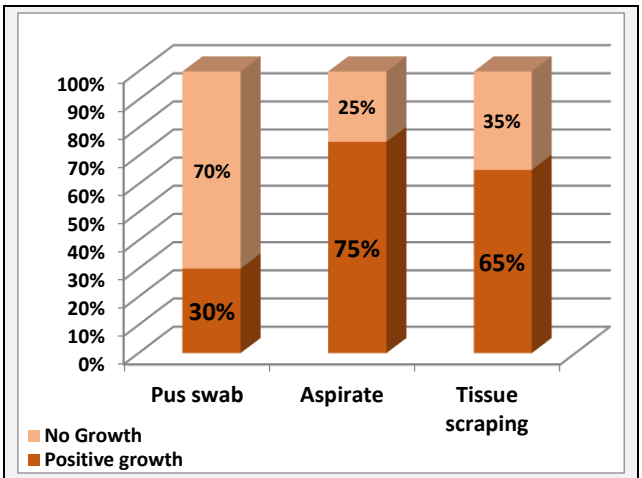

Figure 1. Growth Finding According to Sample Types

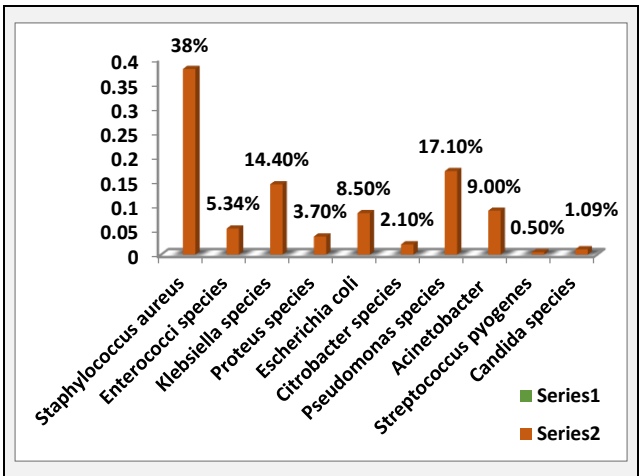

Figure 2. Microbial Profile of Wound Isolates

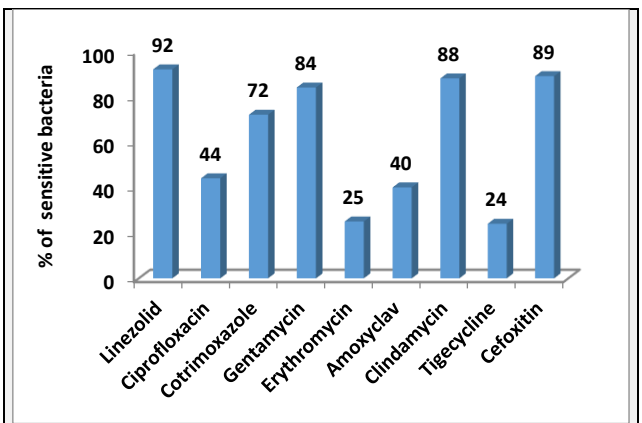

Figure 3. Antibiogram of Staphylococcus aureus

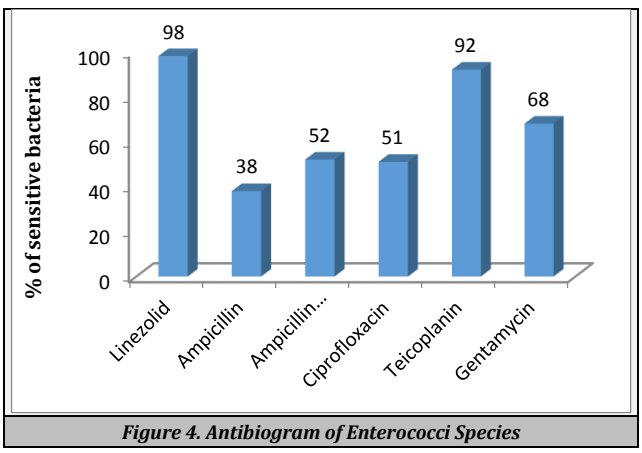

Figure 4. Antibiogram of Enterococci Species

Antibiotic	*E. coli*	*K. pneumoniae*	*Acinetobacter spp.*	*Proteus spp.*	*Pseudomonas spp.*
Pi	44	68	78	79	60
Ak	88	80	70	80	67
Gen	77	84	73	78	67
CPZ	40	76	60	79	94
CFS	80	80	80	82	100
PIT	82	80	100	84	67
COT	65	72	80	60	94
NET	95	75	87	87	100
TGC	100	95	94	100	100
AMC	40	60	75	20	87
CIP	42	70	73	58	77
CTR	36	65	73	62	94
MRP	92	80	93	100	94
IPM	80	79	93	58	53
GAT	-	-	-	-	67
CAZ	-	-	-	-	47
CAC	-	-	-	-	47
AZ	-	-	-	-	60

Table 1. Percentage (%) of Sensitive Strain of different Gram-Negative Bacteria to Different Antibiotics

AK=Amikacin, Pi=Piperacillin, Gen=Gentamycin, CPZ=Cefoperazone CFS= Cefoperazone sulbactam, PIT=Piperacillin tazobactam, COT=Cotrimoxazole Net=Netilmicin, TGC=Tigecycline, AMC=Amoxiclav, CIP=Ciprofloxacin, NA=Nalidixic acid. NX=Norfloxacin, CTR=Ceftriaxone, MRP=Meropenem, IPM=Imipenem, NIT=Nitrofurantoin, GAT=Gatifloxacin, CAZ=Ceftazidime, CAC=Ceftazidime clavulanic acid AT= Azithromycin

Among positive samples 120 (64%) were from Male and 67 (36%) were from female population. Most frequent sample received was pus swab (70%) followed by pus aspirate (25%) and tissue scraping (35%). Growth finding according to sample types was showed in Fig 1. Among 187 positive samples 5.34% (10/187) were from age group 1-20,36% were from age group 21-40.37. 40% were from age group 41-60.21.26% were from age group more than 60. Highest number of positive samples were received from Surgery department (32%) followed by Orthopaedic (27.5%) and Medicine (24.50%), ENT (10%). Microbial profile of wound isolate was showed in Fig 2. Antibiogram of *Staphylococcus aureus* was showed in Fig 3. Antibiogram of *Enterococci* was showed in Fig. 4. Antibiogram of Gram-negative bacteria was showed in Table 1.

DISCUSSION

Wound infection is one of the commonest causes of patient disability and long hospital staying. In severe wound infection patient may die due to sepsis particularly if the bug is multiple drug resistant. Our study was an attempt to diagnose etiological agents and their antibiotic susceptibility pattern in pus sample, received in a tertiary care hospital of Western Odisha. 65% of total sample was positive for any bacteria in our study. This finding was consistent with study like Razina et al[10] (66.2%) and Shamanna et al[11] (65.86%) but it was higher than study done by Rugina et al[3] (60%) Jamatia et al[6] (49.02%), Bhatta et al[12] (60%) Roy et al[13] (50.73%), Gupta et al[14] (62.8%) and lower than study done by Goel et al[15] (71.5%), Bankar et al[16] (78.5%), Rao et al (89.47%), Sharma et al[17] (83%). In positive samples Male and female ratio was 1.7:1 which was higher than Razina et al[10](1.3:1),Bankar et al[16] (1.5:1), Rao et al[17] (1.4:1), Sharma et al[18] (1.2:1) but lower than Duggal et al[4] (2.63:1), Bhalla et al[5] (1.94:1) Goel et al[15] (5.6:1), Kotgire et al[18] (2.5:1). In our study Middle age

group (21-60) was most affected age group like Bhalla et al,[5] Bankar et al[16] and Biradar et al[19]. Highest number of no growth was observed in pus swab (70%) followed by tissue scraping (35%). This finding suggested that pus was a better sample for isolation of causative organism than pus swab. It was supported by study done by Bhalla et al.[5] Pus swab easily gets dried and bacteria in pus swab are killed during transport. Tissue scraping if collected from inappropriate site may give no growth. Highest number of positive samples were received from Surgery department (32%) followed by Orthopaedic (27.5%) and Medicine (24.50%), ENT (10%). Almost similar finding was reported by Razina et al[10] (surgery dept. 33.5%> orthopaedics dept. 21.1%>medicine dept. 19.7%>ENT 7.2%). Bankar et al[16] (surgery dept. 68.09%>orthopaedics dept. 10.2%>OBG dept. 8.2%>medicine dept. 6.25%) and Rao et al[17] (surgery 35.29%> Orthopaedics 20.42%>OBG 11.76%>medicine 9.8%) showed similar pattern. Anshu et al[20] showed slight variation as they had found Skin & VD dept. was the second most common department after Surgery department.

In our study most common bacteria isolated was *Staphylococcus aureus* (38%) followed by *Pseudomonas* species (17.10%), *Klebsiella* species (14.4%), *Acinetobacter* species (9%) and then *E. coli* (8.5%). Least common bacteria were *Streptococcus pyogenes* (0.5%). Bhalla et al,[5] Anshu et al,[20] Sowmya et al,[21] Ananth et al[22] and Kumar et al[23] also showed that *Staphylococcus aureus* was the most common bacteria isolated. Jamatia et al,[6] Shamanna et al,[11] Bessa et al,[24] O. J et al[25] and Tiwary et al[26] showed similar pattern (*Staphylococcus aureus* > *Pseudomonas* species) like our study. But Bhalla et al[5] and Anshu et al[20] showed that *E. coli* was the 2nd most common bacteria unlike our study. Duggal et al[4] showed *Pseudomonas* as most common bacteria. Bhatta et al[12] and Sharma et al[18] showed *Klebsiella* as the most common bacteria. Hospital hand hygiene practice and environmental cleaning should be monitored by Hospital infection control team to reduce Staphylococcal and Pseudomonal infection in wound.

In antibiotic sensitivity *Staphylococcus aureus* showed high sensitivity to linezolid (92%), cotrimoxazole (72%), gentamicin (84%), clindamycin (88%) and cefoxitin (89%). Linezolid sensitivity was very much similar to study done by Duggal et al[4] (94.8%) but not with other studies like Rugina et al[3] (100%) Bhalla et al[5] (100%), and Razina et al[10] (100%) and Samna et al[27] (100%) where almost 100% bacteria were sensitive to linezolid. However, Bankar et al[16] showed 97.1% sensitivity to linezolid. In our hospital linezolid resistance rate was comparatively high because of inappropriate use of linezolid by local practitioners (outside our hospital) as first line therapy in any infection due to availability of oral formulation.

Among all isolates of *Staphylococcus aureus* 11% isolates were MRSA that was lower in comparison to Bhalla et al[5](78%), Razina et al[10] (15.4%), Biradar et al[20] (26.9%), Kshetry et al[28] (37.6%), Sanjana et al[29] (39.6%), Dibah et al[30] (46.3%), Tiwary et al[31] (69.1%),.Difference in MRSA finding was most likely due to difference in the level of irrational antibiotic use, level of environmental hygiene maintained in different hospitals, and level of effective implementation of antibiotic stewardship programme. 75% resistance rate was seen in *Staphylococcus aureus* against erythromycin that was very much higher compared to other studies like Bhalla et al[5] (62%), Jamatia et al[6] (45.29%) Bankar et al[16] (50%), Kotgire et al[19] (25%).In our study clindamycin showed good sensitivity(88%)which was very much higher than Bhalla et al[5] (55%).Good sensitivity was also noted in cotrimoxazole (72%) compared to Bhalla et al[5] (52%), Sharma et al[17] (25%) and Biradar et al[19] (67.3%), Low sensitivity to fluoroquinolones and ß lactam antibiotic (<50%) and good sensitivity towards aminoglycoside (84%) was comparable with another study like Rugina et al[3] Duggal et al,[4] Bhalla et al,[5] Jamatia et al,[6] Rozina et al,[10] Bankar et al,[16] Sharma et al.[17] In case of *Enterococci*, good sensitivity (90%) was seen in case of linezolid, teicoplanin and aminoglycoside but not with ampicillin, ampicillin- sulbactam, ciprofloxacin (<60%) like Bhalla et al.[5]

Non-fermenter gram negative bacteria like *Acinetobacter* species and *Pseudomonas* species showed good sensitivity to piperacillin- tazobactam, cefoperazone, cefoperazone-sulbactam, cotrimoxazole, netilmicin, tigecycline, amoxiclav, ciprofloxacin, 3rd generation cephalosporin and carbapenem (>75%) that was a outcome of effective implementation of antibiotic policy in our hospital. In our study amoxiclav, fluoroquinolones and third generation cephalosporin resistance was very much higher in *E. coli* than *Klebsiella* spp and it was a reverse finding compared to Jamatia et al[6] and Sharma et al[18] but was similar to Rugina et al.[3] It may be due to higher prevalence of *E. coli* infection compared to *Klebsiella* infection in our hospital locality and thereby more antibiotic pressure to *E. coli* strain. *Citrobacter* species showed resistance to 3rd generation cephalosporin and cotrimoxazole and imipenem but high sensitivity to aminoglycoside, cefoperazone-sulbactam, piperacillin-tazobactam, netilmicin, tigecycline, amoxiclav, meropenem that was similar to Rugina et al.[3] *Proteus* species showed good sensitivity against all antibiotic except amoxiclav that was again similar to Rugina et al.[3] Overall all gram negative bacilli showed good sensitivity to carbapenem, aminoglycoside and lower sensitivity to cotrimoxazole, fluoroquinolones, 3rd generation cephalosporin like Duggal et al[4] Jamatia et al.[6] Rozina et al,[10] Balan et al.[32]

CONCLUSIONS

This study showed that the most common organism causing wound infection was *Staphylococcus aureus* followed by *Pseudomonas* species, *Klebsiella* species and *Acinetobacter* species. Linezolid, aminoglycoside and carbapenem could be used as empirical therapy for treating these infections. However, continuous surveillance of antibiotic sensitivity pattern needs to be done to determine the true burden of antibiotic resistance in the community and prevent outbreak by implementing proper antibiotic policy. This study also showed that pus aspirate was better sample than pus swab.

Limitation

Anaerobic culture was not performed in our study due to lack of resource.

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