Original article

Evaluation of microbiological profile and antibiogram of aerobic bacteria isolated from pus samples

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Abstract
Purulent wound infections are quite complicated to manage because of multidrug resistant bacteria. The main purpose of the present study was to assess the prevalence, microbiological profile and antibiograms of aerobic bacteria isolated from pus samples. One hundred pus samples sent to the microbiology laboratory from surgical departments over a period of six months were analyzed. Isolation and detection of culture isolates was done by using standard bacteriological techniques and antibiotic susceptibility testing was performed by disc diffusion method by following CLSI guidelines on Muller-Hinton (MH) agar. Highest number of pus samples were from incision and drainage (23.8%) followed by chronic non-healing ulcer (19.04%). Twenty one different bacterial isolates were obtained from one hundred pus samples. S. aureus was the predominant bacteria (28.5%) followed by coagulase-negative Staphylococci (23.8%). The results of the antibiotics susceptibility testing illustrated that majority of the isolated organisms were MDR. S. aureus showed highest sensitivity to antibiotics like linezolid (83.3%) and teicoplanin (50%). Among the five isolated strains of coagulase-negative Staphylococci (CONS), three of them were MDR and the other two showed sensitivity to antibiotics cefaperazone, co-trimoxazole and ticarcillin/clavulanic acid (20%). Among the 9 isolates of Enterobacteriaceae, one isolate of E. coli (11.1%), two isolates of Klebsiella species (22.2%) showed ESBL production. The isolated four strains of P. aeruginosa showed ESBL production (44.4%) by CAZ/CA antibiotic susceptibility testing. These organisms were screened for carbapenemase production through Modified Hodge test, 5 strains possess carbapenemase enzyme production (71.4%) and thus acquire resistance to carbapenem antibiotics. Carbapenemase production was not detected in the 2 isolates of P. aeruginosa among the 4 tested strains. The overall results showed that 85% of the isolated strains showed MDR to certain classes of antibiotics.

Key words: Antibiotic susceptibility, Carbapenemase production, ESBL detection, Multidrug resistance, Wound infection

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Purulent wound infections account for nearly 17% of nosocomial infections but cause up to 7-10 extra post-operative hospital days stay and from $3000 to $29,000 in extra costs depending on the operative procedure and usually appear 5-7 days after surgery. Due to this reason, many of the surgical practices are now dealing on an outpatient basis and it is more intricate to evaluate the prevalence of wound infections. These infections are typically caused by the patient’s own endogenous flora or hospital acquired skin and mucosal organisms and rarely due to airborne transmission of skin squames that get rid of into the wound from persons in operation theatre. Usually the risk factors for post operative wound infection are associated with surgeon’s technical skill, the patient’s co-morbidities (e.g. Diabetes mellitus, Obesity) or inapt timing of antibiotic prophylaxis and infirmity of the age. The other risk factors are the presence of drains, extended preoperative hospital stays, shaving of the operative site by razor the day before surgery, prolonged period of surgery, and infection at inaccessible sites (e.g. untreated urinary tract infection).

An extensive research interrelated to risk factors for pus oriented infections and the predictable morbidity and financial charges of these infections have guided to national prevention efforts e.g. The Surgical Care Improvement Project (SPIP) and approved for bundling of evidence based preventive measures were issued by the Centers for Medicare and Medicaid Services (CMS) in cooperation with the CDC. Supplementary measures comprised of attention to technical surgical issues and aseptic methods of operating theatres in addition to preoperative therapy for acute infection. Reporting of Surveillance outcomes to surgeons have been related to the decline of infection rates. The practice of diagnosing and treating wound infections starts with a careful assessment of the operating site in the febrile post operative patient. The mortality rate is high >60% when treatment is delayed.

The microorganisms like coagulase-negative Staphylococci, Staphylococcus aureus, Enterococci, Clostridium, Beta-hemolytic Streptococci etc. are frequently associated with purulent wound infections. Treatment of pus oriented infections requires source control drainage or surgical excision of infected or necrotic material and antibiotic therapy aspired at the plausible or laboratory confirmed pathogens. Superficial infections are less likely to produce fever than infections with deep tissue involvement and usually managed with debridement only whereas deep seated infection is dependent on the character of the infection. Localized collections like abscesses can often be managed with drainage only, while more diffuse involvement of deep tissues should have prompt antimicrobial therapy. Now-a-days pus related infections are tricky to manage due to multidrug resistant (MDR) bacteria due to widespread use of prophylactic and empiric antibiotics, increased severity of illness, and greater numbers of immune-compromised patients undergoing surgical procedures.

The main purpose of the present study was to assess the prevalence, microbiological profile and antibiograms of aerobic bacteria isolated from pus samples.

Materials and methods

One hundred pus samples sent to the microbiology laboratory from surgical departments over a period of six months (October 2015 to March 2016) were analysed. The pus samples were collected from infected wounds (Fig 1). Patients were from both sexes and all age groups. Gram staining was carried out initially to study the morphological characteristics of the clinical isolates.

The pus samples were streaked on blood agar, MacConkey agar and Mannitol salt agar plates and incubated aerobically at 37°C for 24h. Colonial morphology provisionally identified the microorganisms and pigment production; the isolated bacteria were analysed by different biochemical tests for further confirmation. Table 1 showed the biochemical tests used for the identification of Gram-negative bacteria. For Gram-positive bacteria coagulase test, catalase test, hemolysis pattern on blood agar, bile esculin hydrolysis for Enterococcus faecalis were used for identification.
Table 1: Biochemical features of Gram negative bacteria

| Tests                        | E. coli | Klebsiella species | Enterobacter species | Citrobacter species | Proteus species | Pseudomonas species |
|------------------------------|---------|--------------------|----------------------|---------------------|-----------------|---------------------|
| Growth on MacConkey agar     | LF      | LF                 | LF                   | LF                  | NLF             | NLF                 |
| Oxidase test                 | -       | -                  | -                    | -                   | -               | -                   |
| Urease test                  | -       | +                  | -                    | -                   | +               | -                   |
| Indole production            | +       | -                  | -                    | -                   | -               | -                   |
| Methyl red                   | +       | -                  | -                    | -                   | +               | -                   |
| Voges-proskauer test         | -       | +                  | -                    | -                   | -               | -                   |
| Citrate utilization test     | -       | +                  | +                    | +                   | +               | +                   |
| Catalase test                | A/A, Gas +, H₂S- | A/A, Gas +, H₂S- | A/A, Gas +, H₂S- | A/A, Gas +, H₂S+ | K/A, Gas +, H₂S+ | K/K, Gas -, H₂S- |
| TSI test                     | Glucose, maltose, mannitol (A/G) | Glucose, sucrose (A/no gas) | Glucose, lactose, sucrose, mannitol (A/G) | Glucose, mannose, lactose, sucrose, mannitol (A/G) | Glucose maltose (A/G) | Mannitol (A/G) |
| Sugar fermentation by the organisms | +   | +                  | +                    | +                   | +               | +                   |

A= Acid, G= Gas, K=Alkaline, H₂S=Hydrogen sulphide, TSI= Triple sugar iron, + = positive, - = negative

**Antibiotic susceptibility testing:** Overnight broth culture of the isolated bacteria was used as inoculums. Antibiotic susceptibility testing was conducted by disc diffusion method based on The Clinical and Laboratory Standards Institute (CLSI) guidelines using Muller-Hinton (MH) agar. The antibiotic discs (HiMedia Laboratories Pvt. Ltd., Mumbai, India) used in this study were piperacillin (100µg), amikacin (30µg), gentamicin (10µg), cefoperazone (75µg), ceftazidime (30µg), ceftriaxone (30µg), ciprofloxacin (5µg), imipenem (10µg), piperacillin/tazobactam (100/10µg), polymyxin B (300U), meropenem (10µg), ampicillin/sulbactam (10/10 µg), levofloxacin (5 µg), colistin (10 µg), doripenem (10 µg), ampicillin (10 µg), amoxycillin (20/10 µg), co-trimoxazole (25 µg), cefepime (30 µg), cefotaxime (30 µg), aztreonam (30 µg), ticarcillin/clavulanic acid (75/10 µg), teicoplanin (30µg), tigecycline (15 µg), linezolid (30 µg), vancomycin (30µg), ceftazidime/clavulanic acid (30/10 µg), cefuroxime (30 µg), penicillin (10 U), erythromycin (15 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), clindamycin (2 µg), doxycycline 30 µg, nitrofurantoin (300 µg). The inhibition zone was measured according to CLSI guidelines (CLSI Catalogue, 2016). Extended spectrum Beta-lactamase confirmation: The production of extended spectrum beta-lactamas by the isolated strains was tested with the CLSI confirmatory test using Ceftazidime (CAZ) (30 µg) disc only and in combination with Clavulanic acid (CA) (10 µg). At least 3cm distance was maintained between the disks. The test is positive if there is an increase in the growth-inhibitory zone just about the CAZ disk with CA was 5 mm or greater of the diameter around the disk containing CAZ alone. The plates were incubated at 37°C for 18h. Detection of Carbapenemase producing organisms by Modified Hodge test: In this test, carbapenemase production by the clinical bacterial isolates was identified if the isolates were able to produce enzyme and permits the growth of stand-
and E. coli ATCC 25922 strain towards a carbapenem disc. The results were noted based on the observation of clover leaf-like indentation. Initially, 0.5 McFarland dilution of the standard strain E. coli ATCC 25922 was prepared in 5 ml of Mueller Hinton broth (MHB). A 1:10 dilution of the culture was prepared by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of MHB broth. A lawn culture was made by streaking an aliquot amount of diluted standard culture on MHA plate and allowed to dry for 5 minutes. Imipenem disc at a concentration of 10 µg was placed in the center of the MHA plate. Now a swab from each test culture sample was streaked from the border of the disc to the rim of the plate\(^1\). Nearly four organisms were streaked on the same plate with one drug. The plates were incubated at 35°C for 24 h.

**Statistical analysis:** Data were statistically analyzed using SPSS software version 20.0. Frequency and percentages were calculated for categorical and ordinal variables. Chi-square test was performed and p value ≤0.05 were considered statistically significant.

**Results**

From the table 2 it was found that, greater part of the pus samples were reported in the age group of 41-60 years (n=10, 47.61%). Fourteen (67%) samples were recorded from males and 7 (33%) from females which indicate male preponderance. This was statistically significant p<0.05. Significant number of samples (p<0.05) were recorded from incision and drainage (23.8%) followed by chronic non-healing ulcer (19.04%) (Table 3).

**Table 2: Distribution of samples by age and sex**

| Age in years | Number (%) | Number of males (%) | Number of females (%) |
|--------------|------------|---------------------|-----------------------|
| 21-40        | 4 (19.04%) | 3 (75%)             | 1 (25%)               |
| 41-60        | 10 (47.61%)| 6 (60%)             | 4 (40%)               |
| 61-80        | 7 (33.3%)  | 5 (71.4%)           | 2 (28.57)             |
| Total        | 21         | 14 (67%)            | 7 (33%)               |

**Table 3: Distribution of samples by type and site of surgery**

| Type of surgery                                           | Site of surgery | No of samples (%) |
|----------------------------------------------------------|-----------------|-------------------|
| Proximal muscle BLAP (Basolateral amygdalar nucleus)     | Limb            | 1 (4.76%)         |
| Appendectomy                                             | Right lower abdomen | 3 (14.28%)       |
| Incision and drainage                                     | Skin            | 5 (23.8%)         |
| Abscesses of left leg                                     | Left side leg   | 1 (4.76%)         |
| Chronic kidney disease (CKD)                             | Middle of the back | 1 (4.76%)       |
| Nasopharyngeal carcinoma (NPC)                            | Nasopharynx     | 1 (4.76%)         |
| Diabetic abscess                                         | Both legs       | 3 (14.28%)        |
| Chronic non-healing ulcer                                 | Leg             | 4 (19.04%)        |
| Orthopedic procedures                                    | Leg             | 2 (9.52%)         |

Intravenous antibiotics were used for pre-operative prophylaxis (Table 4). Out of one hundred pus samples collected from different surgical wound sites, aerobic or facultative anaerobic bacteria were screened from 21 pus samples and the remaining samples were sterile. Table 5 shows that the most prominent microorganism causing surgical site infections (SSIs) in the present study was Staphylococcus aureus followed by coagulase-negative Staphylococci and Pseudomonas aeruginosa (Fig 2).

The results of the antibiotics susceptibility (Table 6) showed that the most prevalent organism Staphylococcus aureus showed 100% resistant to antibiotics like penicillin, cefoxitin, erythromycin, clindamycin, nalidixic acid, ciprofloxacin, chloramphenicol, doxycycline and co-trimazazole (17%), gentamycin (17%), amikacin (17%), vancomycin (33%), teicoplanin (50%). Most of the isolates of CONS were MDR isolates. One strain of Klebsiella species and P. aeruginosa were completely multi-drug resistant. The results showed that 85% of the
bacterial isolates were multidrug resistant to three or more classes of antibiotics. Among the 9 isolates of Enterobacteriaceae, one isolate of E. coli (11.1%), two isolates of Klebsiella species (22.2%) showed ESBL production. All the four isolates of P. aeruginosa showed ESBL production (44.4%) by CAZ/CA antibiotic susceptibility testing (Fig 3).

Table 4: Pre-surgical antibiotic prophylaxis

| Type of surgery                        | Antibiotic prophylaxis          |
|----------------------------------------|---------------------------------|
| Proximal muscle BLAP (Basolateral amygdalar nucleus) | Cefotaxime 1gm                  |
| Appendectomy                           | Cefotaxime 1gm                  |
| Incision and drainage                   | Cefotaxime 1gm                  |
| Abscesses of right leg                  | Cefotaxime 1gm                  |
| CKD (Chronic kidney disease)            | Teicoplanin 400mg               |
| Nasopharyngeal carcinoma                | Piperacillin + Tazobactum 4.5gm |
| Diabetic abscess                        | Cefotaxime 1gm                  |
| Chronic non-healing ulcer               | Cefotaxime 1gm                  |
| Orthopedic procedures                   | Cefotaxime 1gm                  |

ESBL production was not identified in Enterobacter species and Citrobacter species. When all these 7 strains were screened for carbapenemase production by Modified Hodge test, the results showed that 5 strains were positive for carbapenemase producers (71.4%) and 2 strains were negative for carbapenemase production (28.6%) (Fig 4) (Table 7). Two strains of P. aeruginosa did not show the carbapenemase production though positive for ESBL production.

Table 5: Prevalence rate of bacterial isolates

| Organisms              | n  | %   |
|------------------------|----|-----|
| Staphylococcus aureus  | 6  | 28.6% |
| CONS                   | 5  | 23.9% |
| Pseudomonas aeruginosa | 4  | 19.1% |
| Klebsiella species     | 2  | 9.6%  |
| Citrobacter species    | 1  | 4.7%  |
| Escherichia coli       | 1  | 4.7%  |
| Enterococci species    | 1  | 4.7%  |
| Enterobacter species   | 1  | 4.7%  |
| Total                  | 21 | 100% |

CONS = Coagulase negative Staphylococci

Fig 2. Bacteria isolated from different surgeries
Table 6: Antibiotics sensitivity (%) pattern of isolated microorganisms

| Antibiotics | Citrobacter species (N=1) | Enterobacter species (N=1) | Klebsiella species (N=2) | E. coli (N=1) | CONS (N=5) | S. aureus (N=6) | P. aeruginosa (N=4) | Enterococcus faecalis (N=1) |
|-------------|---------------------------|---------------------------|--------------------------|---------------|-----------|----------------|---------------------|-----------------------------|
| Penicillin  | -                         | -                         | -                        | 0.0%          | 0.0%      | -              | -                   | -                           |
| Ampicillin  | 0.0%                      | 0.0%                      | 0.0%                     | 0.0%          | 0.0%      | -              | -                   | 0.0%                        |
| Amoxicillin/sulbactam | 100%                   | -                         | 0.0%                     | 0.0%          | 0.0%      | -              | -                   | -                           |
| Amoxycillin+clavulanic acid | 100%                   | 0.0%                      | 50%                      | 100%          | 0.0%      | -              | -                   | 0.0%                        |
| Amikacin    | 0.0%                      | 0.0%                      | 0.0%                     | 0.0%          | -         | 17%            | 0.0%                | -                           |
| Aztreonam   | 100%                      | 0.0%                      | -                        | -             | -         | 0.0%           | -                   | -                           |
| Cefoperazone| 100%                      | 0.0%                      | 0.0%                     | 0.0%          | -         | 0.0%           | 0.0%                | -                           |
| Cefotaxime  | 100%                      | 0.0%                      | 0.0%                     | 0.0%          | 0.0%      | 0.0%           | -                   | -                           |
| Ceftazidime | 100%                      | 100%                      | 0.0%                     | 0.0%          | 0.0%      | 0.0%           | 0.0%                | 0.0%                        |
| Ceftazidime+clavulanic acid | 100%                   | 100%                      | 0.0%                     | 0.0%          | 0.0%      | -              | -                   | 0.0%                        |
| Ceftriaxone | 100%                      | 0.0%                      | 0.0%                     | 0.0%          | 0.0%      | -              | -                   | 0.0%                        |
| Cefepime    | -                         | 0.0%                      | 0.0%                     | 0.0%          | 0.0%      | -              | 0.0%                | -                           |
| Ciprofloxacin| 100%                      | 0.0%                      | 0.0%                     | 0.0%          | 0.0%      | 0.0%           | 0.0%                | 50%                         |
| Chloramphenicol | -                        | -                         | -                        | -             | 0.0%      | -              | -                   | -                           |
| Clindamycin | -                         | -                         | -                        | -             | 0.0%      | -              | -                   | -                           |
| Colistin    | 0.0%                      | 0.0%                      | 50%                      | 0.0%          | -         | -              | 0.0%                | -                           |
| Co-Trimoxazole | 100%                  | 0.0%                      | 0.0%                     | 0.0%          | 20%       | 17%            | -                   | 100%                        |
| Doripenem   | 0.0%                      | 0.0%                      | 50%                      | 100%          | 0.0%      | -              | 0.0%                | -                           |
| Doxycycline | -                         | -                         | -                        | -             | 0.0%      | -              | -                   | -                           |
| Erythromycin| -                         | -                         | -                        | -             | 0.0%      | -              | -                   | -                           |
| Gentamycin  | 0.0%                      | 0.0%                      | 0.0%                     | 100%          | 0.0%      | 17%            | 0.0%                | 100%                        |
| Imipenem    | 0.0%                      | 0.0%                      | 50%                      | 100%          | 0.0%      | -              | 0.0%                | 100%                        |
| Levofloxacin| 0.0%                      | 0.0%                      | -                        | -             | 0.0%      | -              | 75%                 | -                           |
| Linezolid   | -                         | -                         | -                        | 0.0%          | 83.3%     | -              | -                   | -                           |
| Meropenem   | 0.0%                      | 0.0%                      | 50%                      | 100%          | 0.0%      | 0.0%           | 0.0%                | 100%                        |
| Nitrofurantoin | -                        | -                         | -                        | -             | -         | -              | -                   | 100%                        |
| Nalidixic acid | -                       | -                         | -                        | -             | 0.0%      | -              | -                   | 0.0%                        |
| Piperacillin | -                         | -                         | 0.0%                     | 0.0%          | -         | -              | -                   | -                           |
| Piperacillin+tazobactam | 0.0%                   | 0.0%                      | 50%                      | 100%          | 0.0%      | -              | 0.0%                | -                           |
| Polymyxin-B | -                         | -                         | -                        | -             | -         | 0.0%           | -                   | -                           |
| Teicoplanin | -                         | -                         | -                        | 0.0%          | 0.0%      | 50%            | -                   | -                           |
| Ticarcillin/clavulanic acid | 100%                     | 0.0%                      | -                        | 20%           | -         | -              | -                   | -                           |
| Vancomycin  | -                         | -                         | -                        | 0.0%          | 33%       | -              | -                   | -                           |
| **Statistics** | $\chi^2=20.00,$        | $\chi^2=20.00,$           | $\chi^2=15.44,$          | $\chi^2=20.00,$ | $\chi^2=14.0,$ | $\chi^2=40.10,$ | $\chi^2=31.86,$ | $\chi^2=13.0,$ |
|              | $p=0.39$                   | $p=0.39$                   | $p=0.63$                 | $p=0.39$      | $p=0.42$   | $p=0.001$      | $p=0.001$             | $p=0.37$                    |
Table 7: Extended spectrum beta-lactamase production and carbapenemase detection by phenotypic tests

| Phenotypic tests          | Total (%) | E. coli species | P. aeruginosa species | Enterobacter species | Citrobacter species |
|--------------------------|-----------|-----------------|-----------------------|----------------------|---------------------|
| ESBL producers           | 77.7      | 11.1            | 22.2                  | 44.4                 | 0                   | 0                   |
| Non-ESBL producers       | 22.2      | 0               | 0                     | 0                    | 11.1                | 11.1                |
| Carbapenemase producers  | 71.4      | 14.2            | 28.6                  | 28.6                 | 0                   | 0                   |
| Non-carbapenemase producers | 28.6    | 0               | 0                     | 28.6                 | 0                   | 0                   |

ESBL: Extended spectrum beta-lactamase

Fig 3. ESBL detection by CAZ/CA antibiotic susceptibility testing

Fig 4. Detection of ESBL and Carbapenemase producers in Gram-negative bacteria
Discussion

Purulent wound infections are exemplified by severe local inflammation, habitually with pus formation caused by several pyogenic bacteria and few fungi. These infections can lengthen the hospital stay, hinder in wound healing, and raises the overall cost and morbidity. The current study showed males preponderance (67%) in wound infections. Men were more prone to wound infections perhaps due to disparities in propensity for skin colonization or other anatomical differences, kind of surgery and incision site. Mama et al had found that infection were more in males than in females. Similar findings were also reported by Taye, Gelaw, Goswami, Amoran. Age is one of the significant factor influencing the occurrence of infection. In the present study majority of the cases was reported in the age group 41-60 years (47.61%) which coincides to the results of previous studies. The impact of high infection rate on these particular age groups might be due to reduction in immunity, low healing rate, increased catabolic processes, extended period of pre-operative hospital stay, presence of co-morbidities like obesity, chronic obstructive pulmonary disease (COPD), diabetes mellitus.

A high prevalence of aerobic or facultative anaerobic bacteria was obtained in our study. This was in line and analogous to the previous literature and validate the significance of aerobes in pus related wound infections. The common bacterial isolates found in the study were S. aureus (28.6%), CONS (23.9%), P. aeruginosa (19%), Klebsiella species (9.6%), Citrobacter (4.7%), E. coli (4.7%), Enterobacter (4.7%), Enterococcus (4.7%), etc. Among the Gram-positive organisms, the major isolate obtained in the study was Staphylococcus aureus (28.6%). Several studies had reported that S. aureus was the common isolate of purulent wound infections worldwide with prevalence rate ranging from 4.6% to 54.4%. S. aureus infection is usually associated with patient’s own endogenous flora and it is a skin and nasal microbial flora, acquired also from contaminated hospital environment, surgical devices or from hands of health care workers. Gram-negative bacteria comprised of 49.6% of all the aerobic bacterial isolates causing pus infections worldwide. Among the Gram-negative organisms P. aeruginosa isolation rate was more in the current study. Masaadeh et al and Sohn et al reported P. aeruginosa was the common Gram-negative bacterial isolate of wound infections. Similar study by Akinkunmi et al who conducted a prospective study to identify the microbial pattern of post-operative wound infections and their antibiotic susceptibility pattern. They isolated S. aureus, Pseudomonas, E. coli, Candida, CONS, Enterococcus. Incidence of enteric microorganisms in wound infections is also due to patient’s normal endogenous microbial fecal flora. It is the clear cause of poor hospital hygiene. More mono-microbial growth of the organisms was identified with pus related infections than polymicrobial growth which correlates the studies of Mama et al, Suchitra et al, Kumar et al. A possible reason for this surveillance is using uneven speciation techniques at different institutions.

The antibiotics susceptibility pattern of Staphylococcus aureus showed that, the isolates showed 100% resistant to penicillin, cefoxitin, erythromycin, clindamycin, nalidixic acid, ciprofloxacin, chloramphenicol, doxycycline and co-trimazolox (17%), gentamycin (17%), amikacin (17%), vancomycin (33%), teicoplanin (50%). However one isolate of S. aureus was completely multidrug resistant to all tested antibiotics. Similar findings were noticed by Mama et al. The organisms showed resistance to multiple classes of antibiotics except gentamycin, norfloxacin, ciprofloxacin, vancomycin and amikacin. S. aureus was the predominant microorganism according to Bhatta and Lakhey; and Mulu. Akhi et al isolated 194 bacterial isolates from hundred samples of pus and isolated the predominant organism P. aeruginosa which was in accordance to the current study because the primary gram negative bacteria isolated in our study was P. aeruginosa (19%). These isolates showed multidrug resistance and sensitivity to antibiotics like ciprofloxacin (50%) and levofloxacin (75%). The second most prevalent organism of the study coagulase-negative Staphylococci are multidrug resistant but few isolates showed sensitivity at least towards antibiotics like cefoperazone (20%) and co-trimoxazole (20%). This organism is a normal flora of the skin. Several studies had reported that this is a common contaminant of wound. The overall MDR of the isolates in the current study was 85% which was in line with previous studies conducted in various parts of the world which showed 85% of MDR isolates. The emergence of multidrug resistant organisms over the past few decades has been considered as an foreseeable genetic response to the strong selective pressure inflicted by antimicrobial chemotherapy which plays a critical role in the emergence of antibiotic resistant bacteria.

Among the 9 isolates of Enterobacteriaceae, 7 isolates of E. coli, Klebsiella species, P. aeruginosa showed ESBL production (77%) and when these strains were screened for carbapenemase produc-
tion through Modified Hodge test, 5 strains possess carbapenemase enzyme production (71.4%) and thus acquire resistance to carbapenem antibiotics like imipenem, meropenem, ertapenem and doripenem. Carbapenemase production was not detected in the 2 isolates of P. aeruginosa among the 4 tested strains. Similar study by Sarma et al reported culture reports of 64% of the patients having microbial infection. The major isolate was S. aureus followed by Enterobacteriaceae members like E. coli, Enterobacter cloacae and Klebsiella pneumoniae which were positive for ESBL production and capable of secreting metallo-beta-lactamase enzymes were remained sensitive to antibiotics like colistin and tigecycline.

The patients received prophylactic antimicrobials like cefotaxime, teicoplanin, piperacillin/tazobactam prior to the surgery in the present study. The recent recommendation for antimicrobial prophylaxis to prevent the pus oriented infections is administration of antimicrobial agent within 60 minutes before the surgical procedure and discontinued soon afterwards. All the cases in our study received preoperative antimicrobial agents more than 6 hours before surgery and almost all patients were treated with antimicrobials after surgery. Many of them were even treated until the day of discharge in an attempt to prevent infection. The antibiotics used were cefotaxime and teicoplanin. However the antibiogram results revealed that the isolated organisms were resistant to these antimicrobial agents. The repeated empirical prescription of these antibiotics for treatment and prophylaxis in our hospital might be responsible for observed high degree of resistance. This condition raises a serious alarm and calls for urgent revision of antibiotic policy and antibiotic prescribing guidelines. Bacteria showed a high degree of resistance for commonly prescribed antimicrobials of our study subjects. Higher resistance of the bacterial isolates towards the antibiotics may also be due to practicing self medication, lack of diagnostic laboratory services or unavailability of guidelines regarding the selection of drugs thereby which lead to inappropriate use of antibiotics.

Conclusion

S. aureus was the prime organism isolated from pus obtained from infected wound in the current study. The study concluded that, most of the isolated aerobic bacteria are resistant to multiple classes of antibiotics. Extensive use of inappropriate antibiotics in empirical therapy can cause emergence of resistant bacteria strains, especially in health care centers. Meticulous surgical techniques, proper sterilization, judicious use of antibiotics, improvement of operation theatre and ward environments, control of malnutrition and obesity, treatment of infective foci and diseases like diabetes, helps to control the morbidity of pus infections.

Limitations of study: The limitations of our study were that, anaerobic bacterial profile and fungal cultures were not done on the samples obtained from various surgical departments.

Conflict of interest: Authors have declared that no competing interests exist.

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