



Antimicrobial Sensitivity Pattern of Bacteria Isolated from Pus Sample Collected from a Private Diagnostic Laboratory in Rangpur District of Bangladesh

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[Received: 12 September 2021; Accepted: 3 November 2021; Published: 1 December 2021]

Abstract

Background: The pattern of pathogens isolated from pus culture and their antibiotic sensitivity reports are very crucial for the management of the patients. **Objective:** The objective of the study was to determine the bacteriological profiles and antimicrobial sensitivity pattern of bacteria isolated from pus cultures. **Methodology:** This retrospective study was conducted in a Private Diagnostic Lab, Rangpur, Bangladesh. The samples were collected from January 2020 to December 2020 for a period of one year. Pus samples were collected from patients in a sterile method and was sent to laboratory section for further procedure. The pus was inoculated on Blood agar media and MacConkey's agar media for culture and identification was confirmed by biochemical tests. Antimicrobial sensitivity was done by Disc diffusion method on Mueller Hinton Agar media. **Result:** A total of 180 pus culture were analysed. Among 180 samples, culture positive was 140(77.8%). The most common age group was 18 to 40 years which was 80(44.4%) cases. Among the isolated bacteria *Staphylococcus aureus* was 34(24.3%), *Escherichia coli* 34(24.3%), *Proteus* species 32(22.9%), *Pseudomonas aeruginosa* 18(12.9%), *Klebsiella pneumonia* 10(7.1%), *Streptococcus species* 6(4.3%). **Conclusion:** In conclusion, the most common isolated bacteria are *Staphylococcus aureus* followed by *Escherichia coli*, *Proteus* species, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, with the resistant to majority of the commonly used antibiotics. [Bangladesh Journal of Infectious Diseases, December 2021;8(2):64-70]

Keywords: Antibiotic; antimicrobial; sensitivity; pus culture; resistance pattern

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Conflict of interest: The author(s) declared no potential conflicts of interest.

Funding agency: No

Contribution to authors: All authors were involved from protocol preparation to manuscript writing.

How to cite this article Huda N, Yusuf MA, Sultana H, Hossain M, Andalib S. Antimicrobial Sensitivity Pattern of Bacteria Isolated from Pus Sample Collected from a Private Diagnostic Laboratory in Rangpur District of Bangladesh. Bangladesh J Infect Dis 2021;8(2):64-70

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Introduction

Pyogenic infections are characterized by local and systemic inflammation usually with pus formation. These may be endogenous or exogenous. A break in the skin can provide entry to the surface bacteria which thereby starts multiplying locally. The body's defence mechanism includes bringing immune cells into the area to fight against bacteria. Eventually, accumulation of these cells produces pus which is a thick whitish liquid¹. The human skin and soft tissue infections (SSTIs) are caused by microbial pathogens during or after trauma, burn injuries, and surgical procedures². Both aerobic and anaerobic bacteria have been implicated in wound infections which commonly occur under hospital environment resulting in significant morbidity, prolonged hospitalization and huge economic burden³⁻⁴.

Coagulase positive *Staphylococcus aureus* has been found to be more dominant organism in pus². Suppuration or pus is caused mainly because of bacterial infection and such bacteria are said to be pyogenic or pus forming. Suppuration is the common sequel of acute inflammation and pus contains inflammatory exudate consisting of dead or living neutrophils². Suppuration or pus is caused mainly because of bacterial infection and such bacteria are said to be pyogenic or pus forming. Suppuration is the common sequel of acute inflammation and pus contains inflammatory exudate consisting of dead or living neutrophils³. Pyogenic infections are a significant group of infections caused by pathogens, exogenously or endogenously, during or after trauma, burns and surgical procedures³. These result in the production of pus. The most common pus producing bacteria are *Staphylococcus aureus*, *Klebsiella* species, *Pseudomonas* species, *Escherichia coli* and *Streptococci* species in which *Staphylococcus aureus* is the most common bacteria that produce pus³.

Wounds are the results 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 injuries⁵. 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 death⁴. Wound infection can be caused by variety of organisms like bacteria, virus, fungi and protozoa and may co-exist as poly microbial

communities especially in wound margins and in chronic wounds⁶. In many cases there is a mixed infection with more than one bacterial species⁷. In pus infection breaking of the host protective layer-the skin and thus disturbing the protective functions of the layer, will induce many cell types into the wound to initiate host response. Infection of the wound is the successful invasion and proliferation by one or more species of microorganisms anywhere within the body's sterile tissues, sometimes resulting in pus formation. According to the nature of the infection is the attachment of microorganisms to host cells and they proliferate, colonize and become better placed to cause damage to the host tissues. Wound can be infected by a variety of microorganisms ranging from bacteria to fungus and parasites.

The gram negative and gram-positive microorganisms. The common gram-positive organisms are the β -hemolytic *Streptococcus*, *Streptococcus pyogenes* and *Staphylococcus aureus*. To protect from microorganism today a best option is antibiotic therapy, now different generation antibiotics are present. The routine use of antibiotics in both medical and veterinary medicine has resulted in wide spread antibiotic resistance and development of antibiotic resistance genes especially within the Gram negative organisms. The capability of surviving a variety of environmental conditions makes it a ubiquitous pathogen allowing it to persist on numerous living and non-living surfaces due to minimal nutritional requirements⁴. The pyogenic infections are either mono-microbial or polymicrobial with an average of 5 to 6 organisms often involved in the infections that are caused by a mixture of aerobic and anaerobic organisms. Among the mono- microbial species, *Staphylococcus aureus* and *Staphylococcus epidermidis* are the most common organisms likely to be encountered followed by Gram-negative bacilli, such as *Klebsiella* species, *Pseudomonas* species, *Escherichia coli*, *Proteus* species, *Citrobacter* species, *Acinetobacter* species, *Enterobacter spp.* and others⁸. This present study was undertaken to determine the bacteriological profiles and antimicrobial sensitivity pattern of bacteria isolated from pus cultures.

Methodology

This retrospective study was done in a Private Diagnostic Lab, Rangpur, Bangladesh. The record was collected from January 2020 to December 2020 for a period of one year. The details of the patients were collected. The pus was cultured in routine laboratory and the antimicrobial sensitivity was

done by phenotypic method. Then pus culture and sensitivity reports collected retrospectively from the records. Pus was collected from the patients with boil, carbuncle, furuncle, skin and soft tissue infection from anywhere of the body and were included as study population. Pus samples were collected using sterile techniques and transferred to sterile containers to avoid contamination. These samples were aseptically inoculated onto Blood Agar, MacConkey Agar media and incubated at 37°C overnight under aerobic condition for 24 hours to 48 hours. Later the organisms were identified by performing Gram staining, biochemical tests, motility test and colony morphology as per standard protocols. Kirby-Bauer's Disk Diffusion method was used to test antimicrobial susceptibility and interpreted by Clinical Laboratory Standard Institution (CLSI) guidelines⁷. All the significant isolates were identified and studied by standard procedures and their antibiotic susceptibility pattern was tested and interpreted according to Clinical and Laboratory Standards Institute (CLSI) recommendations, with the automatic identification system against amoxicillin (30 mcg), amoxyclove (20/10 mcg), amikacin (30 mcg), gentamicin (10 mcg), azithromycin (30 mcg), vancomycin (30 mcg), cefaclor (30 mcg), ciprofloxacin (5 mcg), ceftriaxone (30 mcg), ceftazidime (30 mcg), cefuroxime (30 mcg), cefixime (30 mcg), cefotaxime (30 mcg), cephradine, (30 mcg), cotrimoxazole (25 mcg), doxycycline (10mcg), nitrofurantoin (30 mcg), penicillin (10 mcg), tetracycline (30 mcg), levofloxacin (5 mcg),

piperacillin/tazobactam (100/10 mcg), meropenem (10mcg), aztreonam (30 mcg), netilmycin (30 mcg), nalidixic acid (30mcg), imipenem(10mcg). The data were entered and analysed using SPSS software version 21.0. Qualitative data were expressed as frequency and percentage; on the other hand, the quantitative data were expressed as mean with standard deviation. The ethical clearance of this study was taken from the local ethics review committee (ERC).

Results

In the present study, a total of 180 pus culture reports were analysed. Out of total 180 pus samples processed, culture was positive in 140(77.8%) and was negative 40(22.2%) samples (Table 1).

Table 1: Frequency of Culture Result Cases

Culture result	Frequency	Percent
Growth Present	140	77.8
Growth Absent	40	22.2
Total	180	100.0

The most common age group was 18 to 40 years which was 80(44.4%) cases and the growth of culture was found in 62(44.3%) cases. However, in 40 to 60 years of age group was 46(25.6%) cases and among these, culture was positive for 39(27.9%) cases. The difference was not statistically significant ($p=0.148$) (Table II) and (Table 2).

Table 2: Relationship of Age group of Study Population and Culture Result

Age Group	Growth Present	Growth Absent	Total	P value
Less than 18 Years	25(17.9%)	13(32.5%)	38(21.1%)	0.148
18 To 40 Years	62(44.3%)	18(45.0%)	80(44.4%)	
40 To 60 Years	39(27.9%)	7(17.5%)	46(25.6%)	
More than 60	14(10.0%)	2(5.0%)	16(8.9%)	
Total	140(100.0%)	40(100.0%)	180(100.0%)	

The culture positivity was found more in male than female. Among 112 male patients culture growth was present in 91(81.3%) cases and the rest 21(18.8%) cases were growth negative. Among 66 female patients, growth was present in 47(71.2%) cases and growth was absent in 19(28.8%) cases. The difference was not statistically significant ($p=0.121$) (Figure I).

Among the 180 culture positive pus samples, *Staphylococcus aureus* was 34(24.3%) followed by *Escherichia coli* 34(24.3%), *Proteus* species

32(22.9%), *Pseudomonas aeruginosa* was 18(12.9%), *Klebsiella pneumonia* 10(7.1%), *Streptococcus pyogenes* 6(4.3%), *Enterococcus* species was 4(2.9%) and *Acinetobacter* species 2(1.4%) (Figure II). The sensitivity pattern showed that *Staphylococcus aureus* was mostly sensitive to doxycycline (31%), azithromycin (30.4%), cotrimoxazole (27.6%) and ciprofloxacin (27.1%) and was 100.0% resistant to Cefotaxime. *Proteus* species was sensitive to penicillin (75%), tetracycline (72.2%) and resistant to cefotaxime (0.0%) and vancomycin (0%). *Pseudomonas* and

Escherichia coli showed 50.0% sensitive to cefotaxime and piperacillin (14.3%) and (42.9%) and 100.0% resistant to penicillin. *Klebsiella* species was sensitive to cefixime (11.5%) and 100.0% resistant to cotrimoxazole, penicillin, piperacillin, tetracycline and vancomycin (Table 3).

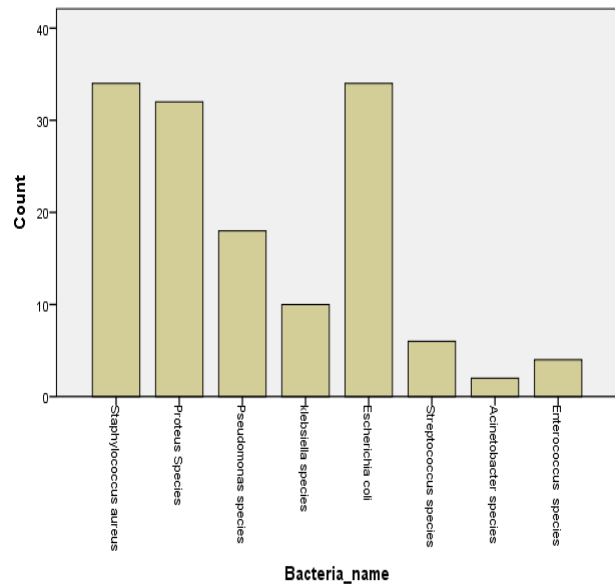
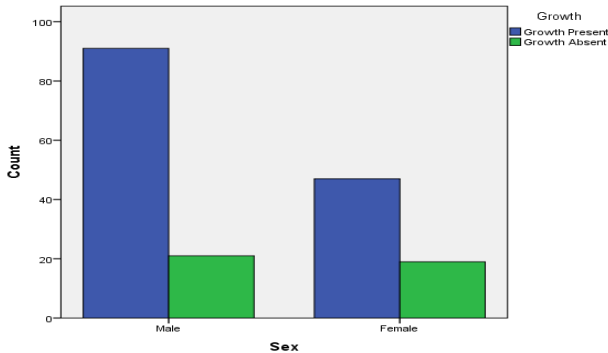


Figure I: Distribution of Gender of Study Population Considering Growth Positivity

Figure II: Bacteria isolated from Pus samples

Table 3: Sensitivity Pattern of Bacteria Isolated from the Pus.

Antibiotic Name	Bacteria name							
	Staph. aureus	Proteus Spp.	Pseudo spp.	Klebsi spp.	E. coli	Strep spp.	Acinato spp	Entero spp
Amoxicillin	16.7%	40.0%	3.3%	6.7%	5	13.3%	0.0%	0.0%
Amoxyclave	21.1%	5.3%	14.0%	8.8%	36.8%	10.5%	0.0%	3.5%
Amikacin	24.8%	21.7%	13.2%	6.2%	24.8%	4.7%	1.6%	3.1%
Azithromycin	30.4%	39.1%	13.0%	8.7%	4.3%	0.0%	0.0%	4.3%
Aztreonam	19.1%	17.0%	14.9%	4.3%	34.0%	2.1%	0.0%	8.5%
Cefaclor	26.8%	32.1%	7.1%	8.9%	16.1%	8.9%	0.0%	0.0%
Cefixime	23.1%	23.1%	7.7%	11.5%	23.1%	11.5%	0.0%	0.0%
Cefotaxime	0.0%	0.0%	50.0%	0.0%	50.0%	0.0%	0.0%	0.0%
Cefuroxime	26.4%	30.2%	11.3%	7.5%	13.2%	11.3%	0.0%	0.0%
Ceftriaxone	26.2%	24.6%	9.2%	6.2%	21.5%	9.2%	3.1%	0.0%
Cephadrine	26.5%	34.7%	10.2%	6.1%	12.2%	10.2%	0.0%	0.0%
Ciprofloxacin	27.1%	20.3%	15.3%	6.8%	20.3%	3.4%	1.7%	5.1%
Cotrimoxazole	27.6%	13.8%	13.8%	0.0%	31.0%	6.9%	0.0%	6.9%
Ceftazidime	19.6%	9.8%	9.8%	3.9%	37.3%	9.8%	3.9%	5.9%
Doxycycline	31.0%	55.2%	3.4%	10.3%	0.0%	0.0%	0.0%	0.0%
Gentamicin	24.7%	28.0%	11.8%	4.3%	21.5%	5.4%	0.0%	4.3%
Imipenem	24.0%	21.5%	10.7%	7.4%	26.4%	5.0%	1.7%	3.3%
Levofloxacin	26.4%	22.0%	13.2%	6.6%	22.0%	4.4%	2.2%	3.3%
Meropenem	26.4%	20.8%	10.4%	8.5%	25.5%	4.7%	0.9%	2.8%
Nalidixic acid	25.0%	28.1%	9.4%	3.1%	25.0%	0.0%	0.0%	9.4%
Netilmicin	24.5%	22.7%	11.8%	6.4%	25.5%	5.5%	0.9%	2.7%
Nitrofurantoin	22.1%	24.4%	12.8%	9.3%	23.3%	4.7%	1.2%	2.3%
Penicillin	16.7%	75.0%	0.0%	0.0%	0.0%	8.3%	0.0%	0.0%
Piperacillin	14.3%	14.3%	14.3%	0.0%	42.9%	0.0%	14.3%	0.0%
Tetracycline	11.1%	72.2%	5.6%	0.0%	11.1%	0.0%	0.0%	0.0%
Vancomycin	20.0%	0.0%	0.0%	0.0%	40.0%	40.0%	0.0%	0.0%

Staph. aureus= Staphylococcus aureus; Pseudo=Pseudomonas; Klebsi=Klebsiella; E. coli=Escherichia coli; Strep=Streptococcus; Acineto=Acinetobacter; Entero=Enterococcus

Discussion

In the present study, a total of 180 pus culture reports were analysed and culture was positive in 140(77.8%) cases and was negative 40(22.2%) cases, which is similar study with Khanam et al¹ 2018 where she found 212 cases aerobic culture, culture was positive in majority cases, which were 131(61.8%) and the rest of 81(38.2%) were growth negative. Roopa and Deepali³ studied 177 samples, 11(6.21%) samples showed polymicrobial growth. The total number of isolates was 188 isolates and gram negative bacteria were isolated more compared to gram positive pathogens. In this study growth culture was positive more among male than female. Among 112 male patients culture growth was present in 91(81.3%) cases and the rest 21(18.8%) cases were growth negative. Again among 66 females, growth was present in 47(71.2%) cases and growth was absent in 19(28.8%) cases. Findings were similar to other authors like Khanam et al¹. It might be due to male works outside more than female and are more prone to injuries and infection³. Considering the age group, culture was positive more in the age group of 18 to 40 years 62(44.3%) followed by 40 to 60 years 39(27.9%), less than 18 years 25(17.9%) and age more than 60 years was 14(10.0%) positive which is similar to Roopa and Deepali³ study.

Among the 180 culture positive pus samples, *Staphylococcus aureus* was 34(24.3%), *Escherichia coli* 34(24.3%), *Proteus* species 32(22.9%), *Pseudomonas aeruginosa* 18(12.9%), *Klebsiella* species 10(7.1%), *Streptococcus* species 6(4.3%), *Acinetobacter* species 2(1.4%) and *Enterococcus* species was 4(2.9%). Rai et al⁹ found *Staphylococcus aureus* (99.0%) was the predominant Gram-positive bacteria isolated and *Pseudomonas aeruginosa* (44%) was predominant Gram-negative bacteria. Rao et al¹ done a similar study and quoted 89.47% of positive aerobic growth, whereas Tameez-ud-Din et al¹⁰ quoted 56% of positive aerobic growth in their study. The highest growth of bacteria from pus culture were *Klebsiella pneumoniae* (13.04%), *Streptococcus anginosus* (10.88%), *Staphylococcus aureus* (8.70%), and also sterile (35.51%). These are similar to the present study result. In another study the most common bacterial isolate was *Staphylococcus aureus* (29.6%) followed by *Escherichia coli* (23.8%) and *Pseudomonas aeruginosa* (14.7%). The antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion technique. *Staphylococcus aureus* was most sensitive to vancomycin (100%) whereas *Escherichia coli* and *Pseudomonas*

aeruginosa showed the highest sensitivity to imipenem (90.7%) and tazocin (80%), respectively¹⁰. Growth was seen in 176 (49.02%) specimens out of 359 samples. A total of 176 specimens yielded single isolate whereas 4 specimens yielded 2 isolates. *Staphylococcus aureus* was the most common organism isolated 53 (30.11%) followed by *Pseudomonas* species 37 (21.02%), *Klebsiella* spp. 30 (17.07%), *Escherichia coli* 24 (13.63%) *Proteus* spp. 11 (6.40%), *Acinetobacter* species 7 (3.97%), *Citrobacter* spp., and *Enterobacter* species 4(2.27%) each. The highest number of multidrug resistant isolates was *Klebsiella* spp. All *Staphylococcus aureus* were 100% sensitive to vancomycin, and all Gram-negative bacilli were 100% to sensitive to imipenem and amikacin¹³. The sensitivity pattern showed that *Staphylococcus aureus* was less than 20.0% sensitive to amoxicillin (16.7%), penicillin (16.7%), piperacillin (14.3%), tetracycline (11.1%). Azithromycin (30.4%) and Doxycycline (31.0%) were more than 30% sensitive. Cefotaxime was 100.0% resistant to *Staphylococcus aureus*. *Proteus* species were low sensitive to Amoxyclave (5.3%), Ceftazidime (9.8%) and Cotrimoxazole (13.8%). *Escherichia coli* was the most prevalent pathogen (51.2%) followed by *Staphylococcus aureus* (21%), *Klebsiella pneumoniae* (11.6%), *Pseudomonas aeruginosa* (5.8%), *Citrobacter* species (3.5%), *Acinetobacter baumannii* (2.3%), *Proteus mirabilis* (2.3%), and *Streptococcus* species (2.3%). *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Citrobacter* isolates were resistant to multiple antibiotics including higher generation cephalosporins. *S. aureus* and *Streptococcus* isolates were sensitive to cloxacillin and vancomycin. However, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Streptococcus* isolates were found to be less resistant to the spectrum of antibiotics tested¹². Tameez-ud-Din et al¹⁰ have reported that the sensitivity test results for antibiotics from pus culture showed the highest sensitive number obtained by meropenem of 73.58%, cefoperazone-sulbactam 69.36%, and oxacillin 66.67%. While the resistance of bacteria to ampicillin antibiotics (54.29%), gentamycin (52.27%), and ampicillin-sulbactam⁸. Pus culture is done to determine the type of bacteria and their sensitivity to antibiotics.

Rao et al¹⁴ study patients underwent pus drainage and received empirical antibiotics in this study. The highest sensitivity test results are meropenem, cefoperazone-sulbactam, oxacillin while the resistance to ampicillin antibiotics, gentamycin, ampicillin-sulbactam and these are similar to the present study¹⁴. Rameshkannan et al¹⁵ studied 114

pus samples received for culture and sensitivity in the microbiology central laboratory, 102(89.47%) cases yielded positive culture while 12(10.53%) cases had no aerobic growth. Among the 102 culture positive pus samples, 97 yielded pure bacterial isolates and 5 yielded mixed infection; so a total number of 107 organisms were isolated out of 102 pus samples. Among the 102 culture positive cases 60 (58.82%) were male and 42 (41.18%) were females yielding a male: female ratio of 1.43. *Staphylococcus aureus* was the most common isolates followed by *Pseudomonas aeruginosa*, *Escherichia coli*, *K. pneumoniae*, *Strept. pyogenes*, *Staphylococcus epidermidis* and *Proteus*. Among the Gram positive isolates, vancomycin, levofloxacin and clindamycin were the most susceptible drugs whereas among the Gram negative isolates, the most susceptible drugs were piperacillin tazobactam, levofloxacin, imipenem and amikacin¹⁴. Out of 264 growth positive samples, Gram-positive bacteria were isolated from 162(61%) samples and Gram-negative bacteria were found in 102(39.0%) samples. Among Gram-positive isolates, 160(99%) were *Staphylococcus aureus* and 2 (1%) were *Streptococcus pyogenes*. Similarly, among Gram-negative isolates, the most prevalent bacteria isolated were *Pseudomonas aeruginosa*, 45(44%), followed by *Klebsiella pneumoniae*, 28 (27%); *Escherichia coli*, 13(13%), *Acinetobacter* species, 7(7%); *Citrobacter koseri*, 4 (4%); *Proteus mirabilis*, 3 (3%); and *Citrobacter freundii*, 2(2%). The bacteriological profile of wound infections among children was similar to that of adults¹⁰. In another study¹¹ the pus culture and sensitivity reports are analysed. *Escherichia coli* (60.7%) was the most common organism isolated followed by *Klebsiella* (20.5%), *Staph. aureus* (9.8%). All isolates were sensitive to meropenem (100%) and piperacillin & tazobactam (89.0%), levofloxacin (65.0%) and amikacin (62.0%). however, high resistance rates were observed with ceftriaxone (70.0%), ceftazidime (64.0%) and cefuroxime (62.0%) in this present study¹.

There are some limitations of the study. Because of resource constraints, it was unable to use molecular level analysis to confirm these results. Further, multicentre study including larger numbers of samples would have generated more significant results.

Conclusion

In conclusion, young adult patients are most commonly subjects of the present study. The most common isolated bacteria are *Staphylococcus*

aureus followed by *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus epidermidis*. This study gives an insight to understand about the common organisms are responsible for wound infection and the antibiotic susceptibility pattern, which is very alarming as it is found that majority of the commonly used antibiotics are resistant. Therefore, rational use of antibiotic should be given to the patients to avoid the antibiotic resistant.

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