Original Article

Antimicrobial Resistance Patterns of Wound/Pus Isolates in a Tertiary Care Setting in Bangladesh.

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Abstract

Introduction: Wound infection is one of the common and important complication of wound. When the infection is serious or the host is compromised, it needs prolong stay in hospital that results significant increase in costs, morbidity, and mortality.

Objective: Bacteria causing wound infection and their sensitivity pattern vary from place to place and it changes frequently over times. Inappropriate use of antimicrobial agents for management of wound infection leads to emergence and spread of drug resistance among the pathogenic bacteria. This study aims to highlights the distribution of bacterial isolates and their antibiotic resistance pattern from wound/pus sample.

Materials and Methods: A retrospective analysis of 425 clinical wound/pus samples were received from patient who were visited in outpatient department and were admitted in TMSS Medical College & Rafatullah community Hospital, Bogura from January 2023 to December 2023. The study was conducted at the Microbiology laboratory of same hospital in Bogura. Bacterial isolates were identified by standard conventional microbial technique and antibiotic susceptibility was carried out by modified Kirby-Bauer disc diffusion method.

Results: Among 425 patients 153 (36%) were aged 21 to 40 years and 152 (35.76%) were 41 to 60 years. Male (58.12%) were predominant than female (41.88%). Growth was seen in 375 (88.23%) samples out of 425. Out of 375 positive samples, 315 (84%) samples yielded single isolates whereas 60 (16%) samples yielded two isolates, and total isolates of bacteria was 435 in number. Among the isolates, Gram-negative bacilli (GNB) 321 (73.79%) were predominant than Gram-positive cocci (GPC) 114 (26.21%). Klebsiella spp. was highest number about 133 (30.57%) followed by Staphylococcus. aureus109 (25.06%), Pseudomonas 99 (22.76%), then E. coli 51 (11.72%). GNB were highly sensitive to piperacillin-tazobactam combination (85%-100%), gentamycin (54%-90%), amikacin (70-85%) and meropenem (68%-100%) and less sensitive to linezolid (05%-20%), vancomycin (0%-35%), 3rd and 4th generation of cephalosporin (10%-40%). Staphylococcus aureus was highly sensitive to co-trimoxazole (82%), vancomycin (74%), linezolid (66%), tigecycline (94%), meropenem (94%), piperacillin-tazobactam combination (95%), amikacin (95%) and less sensitive to ceftazidime (17%), colistin (13%), and aztreonam (14%).

Conclusion: The study revealed the most common organism in pus sample was Klebsiella spp. followed by Staphylococcus aureus than Pseudomonas. So, empiric use of antibiotics based on current antibiogram of wound infection of this hospital and strictly monitoring antibiotic stewardship programs in the hospital.

Keywords: Gram-negative bacilli, Gram-positive cocci, Antibiotic sensitivity test.

Introduction

Wound infection is a common problem in hospitals throughout the world and is caused mainly by bacteria[1] A break in skin or other body tissues caused by injury or surgical incision leads to wound. When bacteria or another pathogen enter, multiply and colonize within the wound, it became infected. These pathogens are either monomicrobial or polymicrobial with mixture of aerobic or anaerobic organisms. Among the monomicrobial 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 others.[2] Infectious diseases are major threat to human health and life.[3] Antibiotics are widely used for their therapeutic and prophylactic purposes but their unselective use in humans and animals combined with improved worldwide connectivity has led to surge in antibiotic resistance. Antibiotic resistance (AMR) especially Gram-negative bacilli have emerged as significant public health problem globally due to insufficient treatment option.[4] During last few decades, multidrug resistance Gram-negative bacterial strains such as Acinetobacter baumanii, E.coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Gram-positive methicillin resistant staphylococcus aureus (MRSA) were increasingly associated with pus infection under hospital setting due to extensive and inadequate dose regimen of antibiotic.[5] 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.[6] Knowing the susceptibility of a certain bacteria to an antibiotic helps you to treat the patient empirically until the culture report is generated. The choice of antibiotics then determined by the results of culture[3]. The control of wound infection has become more challenging due to widespread bacterial resistance to antibiotics. Hospital acquired wounds are among the leading nosocomial cause of morbidity and increasing medical expense.[7] Improving use of antibiotics through antibiotic stewardship is a key to curb the further emergence and spread of antimicrobial resistance (AMR). It is also important for ensuring appropriate treatment.[8] Hence the current study aimed to detect and identify the bacteria from wound infections as well as to determine the antibiotic susceptibility pattern of these isolates at microbiology department of TMC-RCH, Bogura.

Materials and Methods

This retrospective observational study was conducted in the microbiology department at TMC-RCH, Bogura from January 2023 to December 2023 for a period of one year. A total of 425 samples of pus and wound swab received from inpatient and outpatient department of both sex and all aged patients in microbiology department for aerobic culture and sensitivity. Dry wound swabs and improperly labelled samples were excluded. The details of patients were collected. The aseptically aspirated specimens of pus were collected in a sterile leak-proof container or in a sterile syringe, and wound swabs were obtained on sterile cotton swabs and were transported immediately in our laboratory. All specimens were inoculated aerobically on MacConkeys agar, Blood agar, and Hi-chrome agar, then incubated at 37±1°C for 24 hours. Hi chrome media is used for direct detection of the growth of both gram-positive and gram-negative bacteria based on different contrasted colour on a single plate. Further identification of isolates was performed by standard microbiological criteria such as colony morphology, Gram staining, and biochemical properties. Antibiotic susceptibility test was done by modified Kirby-Bauer disc diffusion method on Mueller Hinton agar according to the guidelines of Clinical and Laboratory Standard Institute. According to CLSI Guideline, the size of zone of inhibition was classified as sensitive, intermediate, and resistant to antibiotic tested. Data were analysed by Statistical Package for Social Science (SPSS) software version 30.0, data were presented as frequency and percentage.

Antibiotic disc (Hi Media Laboratories Pvt. Limited, India) such as amoxiclav (20/10mcg), flucloxacillin (30mcg), azithromycin (15mcg), ciprofloxacin (5mcg), levofloxacin (5mcg), vancomycin (30mcg), linezolid (30mcg), tigecycline (15mcg), cefuroxime (30mcg), ceftriaxone (30mcg), ceftazidime (30mcg), cefepime (30mcg), amikacin (30mcg), gentamicin (10mcg), piperacillin-tazobactam (100/10mcg), colistin (10mcg), co-trimoxazole (25mcg), meropenem (10mcg), aztreonam (30mcg) were used for antibiotic susceptibility test.

Results

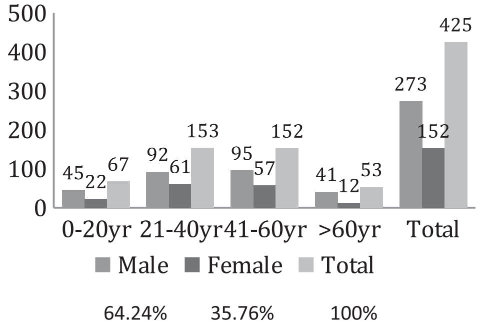


Figure 1: Age and gender distribution of the Study population

Among 425 patients 153 (36%) were aged from 21 to 40 years followed by 152 (35.76%) ranges from 41 to 60 years, 67(15.76%) cases were aged below 20 years and remaining 53 (12.47%) patients were above 60 years of age, male (58.12%) was predominant than female (41.88%) (fig-1). In general, females have stronger innate and adaptive immune response than males.9 Also males are usually engaged in relatively risky and outdoor activities that may the cause of increase infection in male.

Table I: Rate of culture positive pus in different category of patients

Category of patients	Total number of Samples (%)	Culture positive (%)
In-Patient Department (IPD)	321(75.53)	286 (76.27)
Out-Patient Department (OPD)	104 (24.47)	89 (23.73)
Total	425 (100)	375 (100)

104 (24.47%) samples were received from out-patient department (OPD) and 321 (75.53%) from In-patient department (Table-I).

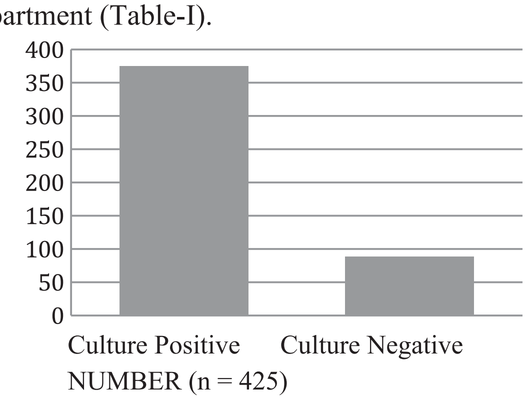
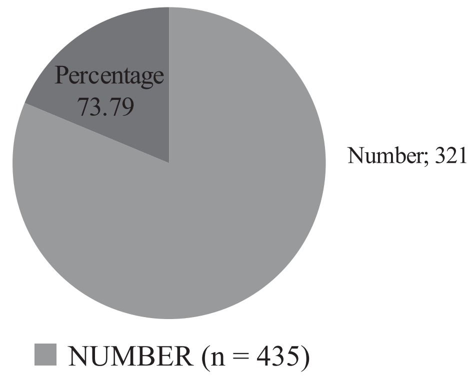


Figure 2: Culture Positive & Culture Negative Pus Samples

Out of 425 samples collected from suspected wound infection, 375 (88.23%) showed growth of aerobic organism whereas 50 (11.77%) showed no growth (fig-2).



GNB (Gram Negative Bacilli), GPC (Gram Positive Cocci)

Figure 3: Number of GNB & GPC among Total Isolates (n=435)

Out of 375 positive samples, 315 (84%) samples yielded single isolate and 60 (16%) yielded two isolates, and total isolated bacteria was 435 in number. Among 435 positive isolates, GNB 321 (73.79%) and GPC 114 (26.21%) (fig -3).

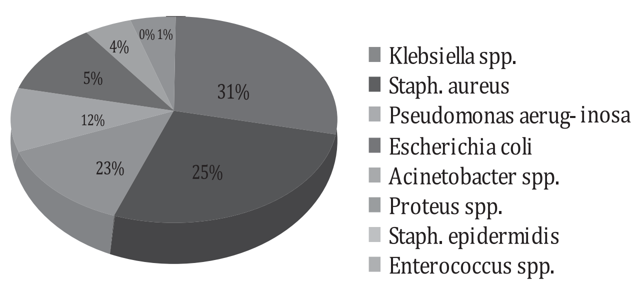


Figure 4: Bacteriological profile of pus samples (n = 435)

Total isolates were 435 bacteria in 375 positive samples. Most common isolated organism were Klebsiella spp. 133 (30.57%) and Staphylococcus aureus 109 (25.06%) followed byPseudomonas99 (22.76%), Escherichia coli 51 (11.72%), Acinetobacter 21 (4.82%), Proteus spp. 17 (3.91%) and Staphylococcus epidermidis 1 (0.23%), Enterococcus 4 (0.92%) (fig-4).

Table II: Frequency and percentage of monomicrobial isolates after aerobic culture from pus/wound sample (n = 315).

Isolated bacteria	Frequency	Percent (%)
Klebsiella spp.	100	31.74
Pseudomonas spp.	75	23.80
E. coli	28	8.89
Proteus spp.	10	3.17
Acinetobacter spp.	13	4.12
Staphylococcus aureus	87	27.62
Staphylococcus epidermidis	01	0.32
Enterococci	01	0.32
Total	315	100

Monomicrobial infections were seen in 315 (84%) samples and polymicrobial infections with growth of two pathogen in 60 (16%) samples. The rate of polymicrobial growth of Pseudomonas spp. and Klebsiella spp. was high (23%) Table II and III.

Table III: Frequency/percentage of mixed isolates (polymicrobial) after aerobic culture from pus/wound sample (n = 60)

Mixed isolated organisms	Frequency	Percent (%)
Pseudomonas spp. + Klebsiella spp.	14	23.33
Pseudomonas spp. + Staphylococcus aureus	01	1.67
Pseudomonas spp. + E. coli	04	6.67
Staphylococcus aureus + Pseudomonas spp.	05	8.33
Staphylococcus aureus + E coli	04	6.67
Staphylococcus aureus + Klebsiella spp.	07	11.67
Staphylococcus aureus + Enterococci	01	1.67
Staphylococcus aureus + Proteus	04	6.67
Acinetobacter+ Klebsiella spp.	03	5.00
Acinetobacter + E coli.	05	8.33
Enterococci spp. + Klebsiella spp.	O2	3.33
E coli + Proteus spp.	03	5.00
E coli + Klebsiella spp.	07	11.66
Total	60	100

Table IV: Antibiotic Sensitivity Pattern of Gram-Negative Bacteria

Antibiotic	Esch. coli (n = 51) (%)	Klebsiella spp. (n =133) (%)	Pseudomonas (n = 99) (%)	Proteus (n = 17) (%)	Acinetobacter (n = 21) (%)
Amoxiclav	45	34	15	37	29
Piperacillin-tazobactam	92	90	90	85	100
Cefuroxime	34	18	10	37	30
Ceftriaxone	40	30	20	39	75
Ceftazidime	30	20	12	36	42
Cefepime	35	38	25	35	71
Ciprofloxacin	55	68	40	45	65
Levofloxacin	52	64	45	30	67
Amikacin	75	85	70	85	82
Gentamicin	70	73	54	82	90
Vancomycin	12	00	00	00	35
Colistin	70	42	78	40	48
Co-trimoxazole	43	68	15	25	95
Meropenem	88	91	68	82	100
Linezolid	05	05	00	10	20
Flucloxacillin	63	12	00	00	41
Azithromycin	35	48	00	00	48
Tigecycline	73	57	28	42	65
Aztreonam	28	25	23	52	25

Among GNB Klebsiella spp. was highly sensitive to piperacillin - tazobactam (90%), meropenem (91%), amikacin (85%), gentamicin (73%) and less sensitive to linezolid (05%), flucloxacillin (12%), cefuroxime (18%), ceftazidime (20%) and fully resistance to vancomycin. Pseudomonas aeruginosa was highly sensitive to piperacillin - tazobactam (90%), colistin (78%), meropenem (68%), gentamicin (54%) and less sensitive to ceftazidime (12%), cefuroxime (10%), Ceftriaxone (20%), amoxiclav (15%), co-trimoxazole (15%), but fully resistant to flucloxacillin, azithromycin, linezolid, vancomycin. E. coli was highly sensitive to piperacillin-tazobactam (92%), meropenem (88%), amikacin (75%) and gentamicin (70%), and less sensitive to linezolid (05%), vancomycin (12%), aztreonam (28%). Acinetobacter was fully sensitive to piperacillin-tazobactam and meropenem, 90% in gentamicin, 82% in amikacin, 67% in levofloxacin, 71% in cefepime, 75% in ceftriaxone, 65% in tigecycline but less sensitive to linezolid (20%), aztreonam (25%). Proteus spp. was 85% sensitive to piperacillin-tazobactam, amikacin and 82% in gentamicin, meropenem and less sensitive to linezolid 10%, but fully resistant to vancomycin, flucloxacillin & azithromycin (Table IV).

Table V: Antibiotic sensitivity pattern of Gram-positive bacteria

Antibiotic	Staph. aureus (n=109) (%)	S. epidermidis (n= 1) (%)	Enterococcus spp. (n=4) (%)
Amoxiclav	68	100	100
Piperacillin - tazobactam	95	100	100
Cefuroxime	56	00	00
Ceftriaxone	62	00	00
Ceftazidime	17	00	00
Cefepime	48	00	00
Ciprofloxacin	63	00	00
Levofloxacin	76	00	00
Amikacin	95	100	00
Gentamicin	90	100	00
Vancomycin	74	100	100
Colistin	13	00	00
Co -trimoxazole	82	100	00
Meropenem	94	00	00
Linezolid	66	100	00
Tigecycline	94	00	00
Flucloxacillin	28	00	00
Azithromycin	24	00	00
Aztreonam	14	00	00

Staphylococcus aureus was highly sensitive to piperacillin-tazobactam (95%), amikacin (95%), gentamicin (90%), tigecycline (94%), co-trimoxazole (82%) but less sensitive to ceftazidime (17%), colistin (13%), aztreonam (14%), azithromycin (24%), flucloxacillin (28%). Staphylococcus epidermidis is fully sensitive to amoxiclav, piperacillin-, amikacin, gentamicin, vancomycin, co-trimoxazole and linezolid and Enterococci spp. was fully sensitive to amoxiclav, piperacillin-tazobactam and vancomycin but fully resistant to all other antibiotic that are used in Table V.

Discussion

In this study, the patients belonging to age group 41 to 60yrs (36.26%) and 21 to 40yrs (34.93%) were found to be higher than other age group. Male (58.12%) was predominant than female (41.88%) (fig-1), that correlate with the studies of Huda et al, Khanum et al, and Deboral et al. This finding could be due to male being more involved in outdoor activities and has the risk of more accidents. Out of 425 cases, aerobic culture was positive in majority cases which was 375 (88.23%) and the rest of 50 (11.77%) cases was growth negative (fig-2) which is nearly to study with Huda et al. Among 375 positive sample, monomicrobial infection was higher 315 (84%), than polymicrobial infection 60 (16%) (Table I). The study by Rajput et al. found that monomicrobial infection was 86% and polymicrobial infection was 10%; this result is consistent with our finding. Polymicrobial wound infections can occur in Bangladesh due to a number of factors, including antibiotic overuse, counterfeit drugs, wound type like large, deep contaminated wound, wound cause like human or animal bite and host factors like immunocompromised host. In present study, the incidence of infection by GNB (73.79%) were predominant in compared with GPC (26.21%). The research was done by Rajput et al (2023), and Khanam et al (2018) also recorded predominance of GNB which was 74.10%, compared with GPC 20.53%, that also corelate with our study. Deboral et al study also showed the predominance of GNB than GNC isolates in pus 2020. In study conducted by Sajjanar et al (2023), found that Staphylococcus aureus was 86 (34.45%), Klebsiella spp.72 (28.8%), Pseudomonas aeruginosa 55 (22%), which is closely similar with our study. Various researchers found that the Staphylococcus aureus was the predominant growth of wound infection, but our study revealed that Staphylococcus aureus is the second highest isolates from pus. Most of the researcher found the predominance of GNB than GPC in their studies. This difference in the distribution of bacterial isolates may be attributed to the difference in the study design, type of lesion, geographical location, and climatic conditions (Deboral et al). In present study, among GNB, Klebsiella spp., Pseudomonas, Esch. Coli, Acinetobacter and proteus are highly sensitive to piperacillin-tazobactam (85%-100%), gentamicin and amikacin (70%-90%), and meropenem (68%-100%) but these organisms are either less sensitive or fully resistant to linezolid and vancomycin, and resistance toward the third and fourth generations cephalosporin is also high such as cefuroxime, ceftriaxone, ceftazidime and cefepime Table IV. Jamatia et al study also recorded the resistance toward the third-generation cephalosporin was high such as cefotaxime, ceftriaxone and ceftazidime. Study done by Rajput et al. showed that Pseudomonas is highly sensitive to gentamicin, meropenem and piperacillin-tazobactam which is synchronized with our study. In Sajianar et al study Pseudomonas is sensitive to gentamycin only in 10.8%, Klebsiella spp. is sensitive to piperacillin-tazobactam only 10.4% and fully resistant to imipenem. Sajjana et al. showed the detected GNB were sensitive to aztreonam (45% to 100%) but present study showed GNB are sensitive to aztreonam 23% to 28% except Proteus spp. (45%). In our study, Staphylococcus epidermidis was fully sensitive to amoxiclav, linezolid, vancomycin and co-trimoxazole that is as like as Sajjanar et al, though Staphylococcus epidermidis is single isolated organism in our study. Staphylococcus aureus was sensitive to tigecycline (94%), amikacin (95%), gentamicin (90%), meropenem (94%), vancomycin (74%), linezolid (66%), co-trimoxazole (82%), levofloxacin (76%), that is nearly to the study of Sajjanar et al, Rajput et al, Deboral et al but less sensitive to ceftazidime (17%), colistin (13%) and aztreonam (14%) Table V. Huda et al and Khanam et al study showed Staphylococcus aureus was less sensitive to vancomycin (18.5-20%), amikacin (0-24.8%), gentamicin (18.6%-24.7%), that is not correlate with our study

Conclusion

In present study, the ranking of bacteria isolated from wound infection are Klebsiella spp.is the first and Staphylococcus aureus is the second position, compared to all other bacterial pathogens. The sensitivity rate of meropenem, piperacillin-tazobactam, amikacin, and gentamicin was high against both gram negative bacilli and gram positive cocci. Due to modification or evolution of bacteria with time and location and irrational use of antibiotics, annual review of microbial flora and their antibiotic sensitivity pattern of this hospital should be carried out for selecting appropriate antibiotic as empiric therapy to reduce the misuse or over use of antibiotic and strictly implementing the antibiotic stewardship programme in hospital settings.

Limitations: The study was done by analysing the retrospective laboratory data only. So one of the limitation of our study was we could not sorting the wound infected patients as complicated or uncomplicated, acute or chronic, also community acquired or hospital acquired. Isolation of anaerobic bacteria could not be carried out in the study due to resource constraints. We are unable to confirm our results using molecular analysis. Large scale study should be conducted to see actual scenario regarding the wound infection.

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Conflict of Interest: None declared.

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