

RESEARCH PAPER

Organisms Isolated from Wound Swab and Pus with their Antibiotic Susceptibility Pattern in a Tertiary Care Hospital of Bangladesh

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Abstract

Background: Antimicrobial resistance is a global health problem and the number of organisms developing resistance to commonly used antibiotics is increasing.

Objective: The study was aimed to find out the pattern of common organism isolated from wound swabs and pus with their antibiogram.

Methods: This observational study was conducted from September 2018 to January 2019 in a tertiary care hospital in Dhaka, Bangladesh. Culture and sensitivity tests were done for wound swabs and pus samples. Data regarding information of the patients, isolated organisms and culture and sensitivity reports were collected from the records of the department of microbiology.

Results: Out of 1709 samples, 72.0% yielded growth of organisms of which 86.4% were gram negative and 13.6% were gram positive bacteria. *Pseudomonas* spp was the most commonly (43.8%) isolated organism from both wound swab and pus samples followed by *Escherichia coli* (16.6%), *Staphylococcus aureus* (11.8%), *Klebsiella* spp (9.8%). Among gram negative bacteria, 14.9% were ESBL producing organisms and *Klebsiella* spp were the most commonly isolated ESBL producers. Most of the bacteria showed high resistance to commonly used antibiotics. Gram negative bacteria were mostly resistant to amoxicillin followed by fluoroquinolones, co-trimoxazole, and cephalosporins whereas colistin, carbapenems and piperacillin/tazobactam were the most effective drugs against them. Majority of gram positive bacteria were resistant to fluoroquinolones and co-trimoxazole but 100% *Staphylococcus aureus* were sensitive to vancomycin, followed by linezolid (98.0%) and teicoplanin (86.0%) and 32.0% of them were Methicillin resistant (MRSA).

Conclusion: The susceptibility pattern shows that some common antibiotics, especially antibiotics of oral form have very limited usefulness in treatment of infections and also highlight the need for regular reporting and antibiogram guided antibiotic prescription.

Keywords: Antibiotic susceptibility, Pus, Resistance, Wound swab

Introduction

Wound infection is one of the most common hospital acquired infections and is an important cause of morbidity and mortality worldwide.¹ Postoperative wound infection delays recovery, increased hospital stay and may produce long lasting sequelae.² Skin and soft tissue infections are caused by microbial pathogens after trauma, burn or surgical procedures result in production of pus.^{3,4} Bacterial infection is a serious problem to the successful treatment of wound

resulting in complications sometimes leading to fatal sepsis.⁵

Spectrum of isolated pathogens usually depends on surgical procedure involved, hospital environment and also the infection prevention policy of the hospital.⁶ Prevalent organisms associated with wound infection and pus formation include *Staphylococcus aureus*, Enterobacteriaceae, *Pseudomonas aeruginosa*, Coagulase Negative Staphylococci (CoNS), *Enterococci*.^{7,8}

Use of antimicrobial agents cause a 'selective pressure' on microbial population.⁹ As a result of indiscriminate use of antimicrobial agents, significant changes occur in microbial genetic ecology.¹⁰ The increasing frequency of antimicrobial resistance among

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pathogens causing nosocomial and community acquired infections is making numerous classes of antimicrobial agents less effective.¹¹

The emergence of antibiotic resistance and its rapid spread among pathogenic bacteria is a grave threat to public health worldwide. During last few decades, multidrug-resistant bacterial strains such as *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, Methicillin resistant *Staphylococcus aureus* (MRSA) are increasingly associated with infections under hospital settings.^{12,13} Extended-spectrum beta-lactamases (ESBLs) are enzymes that mediate resistance to extended spectrum e.g., third generation cephalosporins and the incidence of ESBLs in gram negative bacteria has increased in recent years.^{14,15}

An in-depth knowledge of the pattern of predominant organisms in pus and wound is essential for the treatment of the patient before getting the result of microbiological culture. This would be crucial to reduce the overall infection related morbidity and mortality. The present study was therefore designed to determine the current microbial spectrum of wound infection with their antibiotic sensitivity pattern.

Materials and Methods

This observational study was conducted in the Department of Microbiology of Dhaka Medical College, Dhaka, Bangladesh for a period of five months from September 2018 to January 2019.

All wound swabs and pus samples from both inpatient and outpatient departments of Dhaka Medical College Hospital sent to the microbiology laboratory for culture and sensitivity test regardless of age, sex and antibiotic intake were included in this study. Data regarding the identity of the patient, referring departments, type of specimen and sensitivity reports were collected from the records of the laboratory.

Microbiological methods

Culture of wound swab and pus: Samples were inoculated in blood agar and MacConkey's agar media and incubated aerobically at 37°C for 24 hours.

Isolation and identification of bacteria: The inoculated plates were examined for bacterial growth and organisms were identified by colony morphology, hemolytic criteria, pigment production, Gram stain and different biochemical tests like catalase test, coagulase test, oxidase test, and reaction in TSI agar, MIU and Simmon's citrate agar media.

Antimicrobial susceptibility test: Antimicrobial susceptibility pattern of isolated organisms were done following Kirby-Bauer disc diffusion method.¹⁶ Sensitivity was done using commercially available anti-biotic discs (Oxoid, UK); amikacin (30µg), amoxycylav (20µg amoxycillin/10µg clavulanic acid), cefoxitin (30µg), ceftazidime (30µg), ceftriaxone (30µg), clindamycin (2µg), ciprofloxacin (5µg), colistin (10µg), doxycycline (5µg), gentamicin (10µg), imipenem (10µg), levofloxacin (5µg), linezolid (30µg), meropenem (10µg), piperacillin/tazobactam (100/10µg), teicoplanin (30µg), vancomycin (30µg). Zone of inhibition was measured according to the CLSI guideline.¹⁴

Detection of ESBL: ESBL production in gram negative bacteria was detected by double disc synergy test following CLSI guideline using amoxycylav and 3rd generation cephalosporins discs.¹⁴

Data management: Collected data were classified according to characteristics and various statistical methods and 'Microsoft Excel' software were used for analysis.

Results

A total of 1709 samples (wound swabs and pus) were cultured. Of them 1083 were wound swabs; among which 846 (78.1%) yielded growth of bacteria and 626 samples were pus of which 385 (61.5%) were positive in culture. Difference between the isolation rate of organisms from wound swab and pus is statistically significant ($p < .001$, Chi-Square test, $df = 1$; 95% CI). A total 1231 (72.0%) samples yielded growth of bacteria (table I).

Among total 1231 culture positive samples 1063 (86.4%) yielded growth of gram negative bacteria and 168 (13.7%) had growth of gram positive bacteria. In

Table-I: Growth of bacteria in different samples

Samples	Growth (%)	No Growth (%)	Total (%)	P value
Wound swab	846 (78.12)	237 (21.88)	1083 (100)	<.0001 ^s
Pus	385 (61.50)	241 (38.50)	626 (100)	<.0001 ^s
Total	1231 (72.03)	478 (27.97)	1709 (100)	

^s -significant. Difference between growth rate of wound swab and pus are statistically significant ($p < .0001$).

wound swabs, 765 (90.4%) were gram negative and 81 (9.6%) were gram positive bacteria; in pus samples, 298 (77.4%) had growth of gram negative and 87 (23.6%) were gram positive bacteria (figure 1).

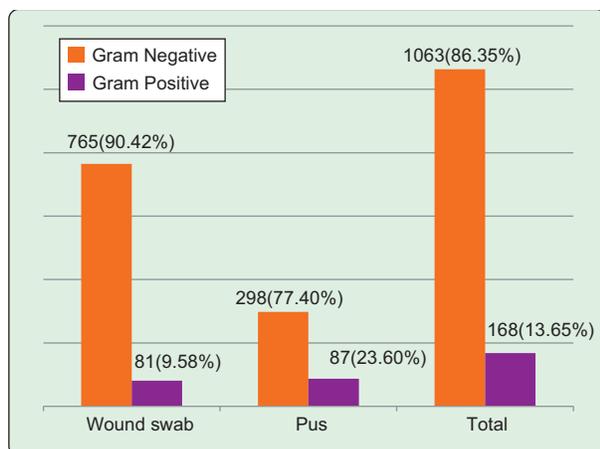


Figure 1: Distribution of isolated bacteria in different samples according to the Gram reaction

Pseudomonas spp was the most commonly isolated organism (43.8%) from both wound swab and pus samples. *Escherichia coli* was isolated from 16.6% samples, 9.8% samples yielded growth of *Klebsiella*

spp and *Proteus* spp was isolated from 7.6% samples. *Staphylococcus aureus* was the most frequently isolated (11.8%) gram positive bacteria from both wound swab and pus (table II).

Among the isolated gram negative bacteria, 14.7% (157/1056) were ESBL producing organisms. *Klebsiella* spp were the most commonly isolated ESBL producers, 32.2% of which produced ESBLs. Among *Pseudomonas* spp 9.5% were ESBLs producer; 19.61% *Escherichia coli*, 15% of *Proteus* spp, 12.3% *Enterobacter* spp and 20% of isolated *Acinetobacter* spp were ESBL producing organisms (figure 2).

Carbapenems were very effective antibiotics against *E. coli*, *Klebsiella* spp. *Proteus* and *Enterobacter* spp; colistin was found most effective antibiotic in vitro against all gram negative except *Proteus* spp. Piperacillin/tazobactam was a very effective antibiotic in vitro against *Enterobacter*, *Proteus* and *Citrobacter* spp (table III).

100% *Staphylococcus aureus*, CoNS and *Enterococcus* were sensitive to vancomycin. *Staph aureus* were also mostly sensitive to linezolid and teicoplanin was very effective antibiotic in vitro against Staphylococci (table IV).

Table-II: Pattern of isolated bacteria from wound swab and pus

Organism	Wound swab Isolates n(%)	Pus Isolates n(%)	Total Isolates n(%)
<i>Pseudomonas</i> spp	431 (50.95)	108 (28.05)	539 (43.79)
<i>E. coli</i>	102 (12.06)	102 (26.49)	204 (16.57)
<i>Stah aureus</i>	68 (8.04)	77 (20.00)	145 (11.78)
<i>Klebsiella</i> spp	89 (10.52)	32 (8.31)	121 (9.83)
<i>Proteus</i> spp	73 (8.63)	20 (5.19)	93 (7.55)
<i>Enterobacter</i> spp	44 (5.20)	21 (5.45)	65 (5.28)
<i>Acinetobacter</i> spp	13 (1.54)	7 (1.82)	20 (1.62)
<i>Citrobacter</i> spp	8 (0.95)	6 (1.56)	14 (1.14)
CoNS	6 (0.71)	7 (1.82)	13 (1.06)
<i>Streptococcus</i>	5 (0.59)	3 (0.78)	8 (0.65)
<i>Serratia</i> spp	5 (0.59)	2 (0.52)	7 (0.57)
<i>Enterococci</i> spp	2 (0.24)	0 (0.00)	2 (0.16)

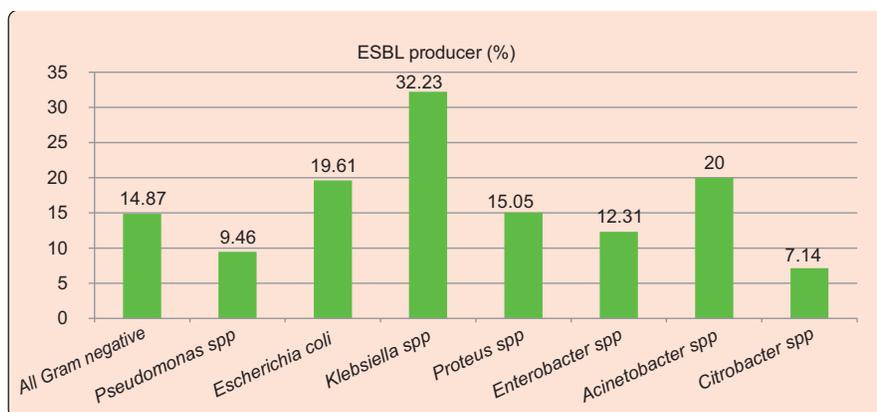


Figure 2: Distribution of ESBL producing gram negative organisms from wound swab and pus.

Table-III: Antibiotic sensitivity pattern of gram negative bacteria isolated from wound swab and pus.

Organism	Sensitivity (%)												
	Ak	Aml	Amc	Caz	Cro	Cip	Cot	L	G	Imp	Mem	Tzp	CT
<i>Pseudomonas</i>	27	-	8	56	9	20	22	11	27	66	68	62	97
<i>E. coli</i>	43	7	10	20	23	21	19	39	41	91	82	71	95
<i>Klebsiella</i>	48	-	9	11	13	18	29	48	30	87	85	65	93
<i>Proteus</i>	33	3	13	19	21	23	42	33	23	89	81	78	4
<i>Enterobacter</i>	45	8	11	23	22	18	45	37	37	97	95	82	97
<i>Citrobacter</i>	57	14	28	28	28	21	50	42	64	86	86	79	100
<i>Acinetobacter</i>	15	-	10	0	10	10	25	15	10	40	35	35	100
<i>Serratia</i>	57	29	29	0	57	0	71	57	71	100	100	100	100

Ak-amikacin, Aml-amoxicillin, Amc-amoxycloclav, Caz-ceftazidime, Cip-ciprofloxacin, Cot-Co-trimoxazole, Cro-ceftriaxone, CT-colistin, G-gentamicin, Imp-imipenem, L-levofloxacin, Mem-meropenem, TZP-piperacillin/tazobactam.

Table-IV: Antibiotic sensitivity pattern of gram positive bacteria isolated from wound swab and pus.

Organism	Sensitivity (%)												
	Ak	Aml	Cro	Cip	Cot	Do	G	L	Lzd	Fox	CD	Tec	V
<i>Stah aureus</i>	41	-	34	20	29	35	39	24	98	68	76	86	100
CoNS	62	-	62	31	38	62	69	38	100	62	69	69	100
<i>Streptococcus</i>	50	88	100	50	50	50	75	63	-	-	-	-	-
<i>Enterococci</i>	0	50	-	100	-	50	0	50	100	-	-	-	100

Ak-amikacin, Aml-amoxicillin, CD-clindamycin, Cip-ciprofloxacin, Cot-Co-trimoxazole, Do-doxycycline, Fox-cefoxitin, G-gentamicin, L-levofloxacin, Lzd-linezolid, Tec-teicoplanin, V-vancomycin.

Discussion

Despite of application of the basic principles in wound care, a number of patients develop infections that require proper identification of the organisms for appropriate management. A changing pattern of isolated organism and their antimicrobial sensitivity which varies from hospital to hospital is a usual feature.

Development and spread of antibiotic resistance can be controlled by appropriate antimicrobial use, strict infection control, and continued surveillance.¹⁰

In the present study, about three fourth samples showed growth of bacteria on culture and it was comparable with growth rate reported in several other studies.^{17,18} In consistent with other studies,^{19,20}

most of the isolated organisms from wound swab (90.4%) and pus (77.4%) were gram negative bacilli. In contrast to the present findings almost equal proportion of gram positive and gram negative bacteria were reported from pus and wound swab in some studies.^{20,21} Pattern of bacteria causing wound infection depends on hospital environment and surgical procedure involved.⁶

Among gram negative bacteria *Pseudomonas* spp was the most commonly isolated organism in this study followed by *E. coli*, *Klebsiella* spp and *Proteus* spp. In accordance, *Pseudomonas* spp was found to be the most common gram negative bacteria in a study in India.²² The frequency of *Pseudomonas* spp. as the causative agent of wound infection was 28% in 2011 and 26.5% in 2016 in Bangladesh, which is lower than this study.^{23,24} This higher rate of *Pseudomonas* infection might be due to the fact that majority of the wound swabs samples were sent from the burn unit of the hospital. *Pseudomonas* spp is a ubiquitous and versatile human opportunistic pathogen and from the endogenous gastrointestinal flora or environmental source it comprises one of the most common causative agents of burn wound infection.^{25,26} *Pseudomonas* produces both cell-associated and extracellular virulence factors that mediate a number of processes, including adhesion, leukocyte killing, tissue destruction, immune system evasion and bloodstream invasion that make it an efficient agent for infection in burn wound.^{27,28} In contrast to the present findings *E. coli* and *Klebsiella* spp were reported as the most predominant gram negative bacterial pathogen.^{18,19,29} The discrepancy of the isolation rate may be due to varying prevalence of infection causing bacteria from hospital to hospital as because different hospital deals with different types of infection.

In this study, *Staphylococcus aureus* was the most frequently isolated gram positive bacteria from both wound swab and pus which was similar to the other studies done in Bangladesh and India.^{17,18,30} About 32% of the isolated *Staphylococcus aureus* were Methicillin resistant *Staphylococcus aureus* (MRSA). In Bangladesh, the rate of MRSA infection ranges from 32% to 63% in different studies which is in accordance to the isolation rate in this study.³¹ MRSA is a multidrug resistant bacterium which is resistant to methicillin and other penicillins and most cephalosporins, β -lactam/ β -lactamase inhibitor combinations and carbapenems.¹⁴

The antibiotic susceptibility data in this study showed that some common antibiotics have very limited

usefulness for treatment of wound infections. Highest resistance by gram negative bacilli was noted against amoxicillin followed by fluoroquinolones, cotrimoxazole, and third generation cephalosporins in our study. This pattern of resistance has been shown by several studies.^{32,33} Ciprofloxacin is an important antibiotic but gram negative bacilli showed high resistance to it. This finding is consistent with studies who reported gram negative bacteria were highly resistant to ciprofloxacin.³⁴⁻³⁶ The higher rate of resistance to ciprofloxacin might be due to the fact that this drug is used widely in Bangladesh for many infections such as enteric fever that is endemic here. Majority of the gram negative bacilli showed a comparatively good sensitivity to amikacin and the pattern is consistent with other studies in Bangladesh.³⁴⁻³⁶ The reason behind such low resistance might be the less use of this antibiotic in this hospital. The result indicates that amikacin may be considered as an alternative drug in infections caused by gram negative bacilli in this setting.

Carbapenems were very effect antibiotics showing 66-97% sensitivity against gram negative bacilli except *Acinetobacter* spp in this study, which is in accordance with findings of several studies.^{34,35} Though only few cases showed resistance but this resistance to carbapenems is a matter of great concern in treatment of infection. Colistin and piperacillin/tazobactam showed higher rate of sensitivity in this study. All isolated *Klebsiella* and *Acinetobacter* spp were sensitive to colistin, and except for *Pseudomonas* and *Acinetobacter* spp piperacillin/tazobactam showed good sensitivity to gram negative bacilli. These two injectable drugs are usually considered as reserve drug and are being used for those who are resistant to most other antibiotics.

In this study, 14.6% of the gram negative bacteria were detected as ESBL producer which was in accordance with a study in 2017.³⁷ In Bangladesh, the rate of ESBL producing bacteria were 23% in 2008 and 24% in 2012 which is higher than the present findings.^{35,38} Reduced use of cephalosporins and comparatively more use of carbapenems and colistin in treatment of infection by gram negative bacteria in present time in this hospital setting may be an important reason for reduced proportion of ESBL producers in the present study. Low rate of ESBL in this study could also be due to inclusion of all indoor and outdoor samples while previous studies were done on infected surgical wound only or ICU patients. *E. coli* and *Klebsiella* spp were the most commonly

isolated ESBL producing organisms in this study. Detection of ESBL-mediated resistance is of great importance because of limited therapeutic options.¹⁵

Gram positive bacteria were mostly sensitive to vancomycin and linezolid. All the *Staphylococcus aureus* were sensitive to vancomycin, followed by linezolid (98%) and teicoplanin (86%); and had reduced sensitivity to ciprofloxacin (20%), levofloxacin (20%) and Co-trimoxazole (29%) which was similar to the sensitivity pattern of *Staph aureus* in a study in 2019 in Bangladesh.³⁹

Overall antibiotic susceptibility data in this study shows that with few exceptions bacteria were found more resistant to antibiotics used in oral form. In Bangladesh, antibiotics especially oral antibiotics are common subjects of indiscriminant uses; antibiotics are sold over the counter in some places and anybody can purchase it even without prescription. Moreover, drug sellers often offer alternative oral antibiotics instead of the prescribed one. There is also lack of awareness among some patients about maintaining the prescribed right dose and duration of antibiotic. As a result of such factors antibiotic resistance is very common in Bangladesh.

Conclusion

There are very limited treatment options available for the resistant bacteria. So, early detection and appropriate antibiotic application remain a significant priority in controlling the development and spread of multidrug resistant organisms. The findings of this study provide an insight to the current state of causative organisms of wound swabs and pus and their sensitivity pattern. An effective national level antibiotic policy along with infection control measures should be introduced to preserve the effectiveness of antibiotics.

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