SUSCEPTIBILITY OF BACTERIAL ISOLATES FROM WOUND SWABS IN BANGLADESH: LABORATORY – BASED SURVEILLANCE STUDY

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Abstract

Background: *Wound infection is one of the major health problems that occur frequently. Infections of the wound result from entry of the organisms through breached skin. It plays an important role in the development of chronicity, delaying wound healing. It is evident that wound infection is a challenging situation for the physicians. Multiple bacteria can cause wound infection. Both broad spectrum and narrow spectrum antibiotics are available for the treatment. It is ideal to give proper antibiotic after culture and sensitivity of the wound swab. Improper and irrational use of antibiotics can lead to drug resistance.*

Objective: *To isolate and identify the bacteria causing wound infection and to determine the antimicrobial susceptibility pattern.*

Materials and method: *This descriptive cross-sectional study was carried out at the Microbiology Laboratory, Popular Diagnostic Centre Ltd, Dhanmondi, Dhaka, Bangladesh, from November 2019 to January 2020. A total of 62 wound swabs were collected and analyzed for culture and antibiotic sensitivity.*

Results: *From total wound swab samples, 49 (79%) were culture positive. The most common isolated pathogen was* Acinetobacter spp. *20 (40.81%). Other isolates were* Staphylococcus aureus *10 (20.41%);* Escherichia coli *07 (14.29%);* Pseudomonas aeruginosa *07 (14.29%); Klebsiella 04 (08.16%) and Proteus 01 (02.04%). Among the Gram negative isolates, Acinetobacter spp showed 100% sensitivity to colistin, 85% to imipenem, 30% to amikacin and 25% to piperacillin + tazobactum.* E. coli *showed 100% sensitivity to imipenem and colistin and least sensitivity to third generation cephalosporin. Pseudomonas showed 100% sensitivity to piperacillin+tazobactum, 85.71% to imipenem and aztreonam. Among the Gram positive isolates,* Staphylococcus aureus *showed 100% sensitivity to linezolid, vancomycin, imipenem, cloxacillin, amikacin and least sensitivity to azithromycin.*

Conclusion: Acinetobacter spp. *was the most frequently isolated pathogen from wound swab and the antibiotic sensitivity pattern of various isolates will help the clinician in appropriate selection of antibiotics against wound infection.*

Key words: *Wound infections, Bacteriology, Antimicrobial susceptibility*

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Introduction:

A wound is a break in the integrity of the skin or tissues, which provides a moist, warm and nutritious environment that is conductive to microbial colonization and proliferation.[1] Development of wound infection depends on the many factors including preexisting illness, length of operation, wound class and contamination.[2]

Wounds presented by patients vary from one setting to another, ranging from acute surgical wounds, traumatic wounds such as those that

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occur following an accident, burn wounds or chronic wounds such as diabetic foot, leg and pressure ulcers.[3]

Infection of the wound is the invasion and proliferation by one or more species of microorganisms sometimes resulting in pus formation.[4] It constitutes a major barrier to healing and can have an adverse impact on the patient's quality of life.[5]

Indicators of wound infection include redness, swelling, purulent exudates, smell, pain, and systemic illness in the absence of other foci. Subtle signs of local wound infection include unhealthy "foamy" granulation tissue, contact bleeding, tissue breakdown, and epithelial bridging.[6]

Wounds infection is often caused by three or more microorganisms. These organisms include Gram positive and Gram negative bacteria as well as both aerobic and anaerobic bacteria species.[7] The prevalent organisms that have been associated with wound infections include *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococci*, *Escherichia coli, Acinetobacter species, Klebsiella pneumonia*, *Proteus* species and *Pseudomonas aeruginosa.*[8] *Staphylococcus aureus*, according to a study have been found for 20-40%. Infection with *Pseudomonas aeruginosa* mainly following surgery and burns account for 5-15%. Other pathogens such as Acinetobacter, Enterococci, *Escherichia coli*, Klebsiella species and Proteus species have been implicated especially in immunocompromised patients and following abdominal surgery.[9] The fungal organisms like Candida species also responsible for wound infection.[10]

Wound care constitutes an important part of routine care given by health professionals to the community population. Advances in control of infections have not completely eradicated wound infection because of development of drug resistance.[11] High resistance of the isolates to antibiotics may be due to practicing self medication, lack of diagnostic laboratory services or unavailability of guidelines regarding the selection of drugs leads to inappropriate use of antibiotics.

This study is undertaken to determine the common bacterial agents associated infected wounds and characterize their antimicrobial susceptibility patterns to the common antibiotics used in therapy. It will be an orientation to health care practitioners who deal with wound management, helping them to choose the appropriate treatment options to control wounds infection.

Methods

Study design and study area

This descriptive cross-sectional study was carried out at the Microbiology Laboratory, Popular diagnostic Ltd, Dhanmondi, Dhaka, Bangladesh, from November'2019 to January' 2020.

Data collection and laboratory procedures

A total of 62 wound swab samples were collected from patients with various wound infections including post-operative surgical wounds, burn wounds and superficial and soft tissue infections. Selective criteria were considered: infected wound and before administration of antibiotics. Swab was collected by gently rolling the swab over the surface of the wound approximately five times, focusing on an area where there is evidence of pus or inflamed tissue. Then the specimens were transported within one hour to the Microbiology laboratory to perform the culture and susceptibility tests.

Bacterial identification

Each specimen was inoculated on Blood agar and MacConkey's agar plate. The culture plates were incubated aerobically at 37°C for 24–48 hours. All the plates were regularly inspected for growth,

Identification of the isolated bacteria was done by colony morphology, Gram-staining and standard biochemical tests.[12]

Antimicrobial susceptibility test

Antimicrobial susceptibility test was carried out by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (MHA) media according to Clinical Laboratory Standards Institute (CLSI) guidelines 27[13] and antibiotic disc was used from OXOID CO. Minimum distance of each disc were 24 mm from center to center of the disc. Zone of inhibition were measured in millimeters after 24 hours of incubation.

Based on the zone of inhibition obtained, the isolates were classified into sensitive and resistant pattern.

For each separate group of organisms separate set of antimicrobials were used. The antibiotics discs and their concentrations were as follows:

Antibiotic disc with concentration	Acinetobacter spp	*E. coli*	Klebsiella spp	Pseudomonas spp	*S. aureus*
Amikacin (30 mcg)	+	+	+	+	+
Ciprofloxacin (5mcg)	+	+	+	+	-
Levofloxacin (5mcg)	+	+	+	+	-
Cephradine (30 mcg)	+	+	+	-	+
Cefoxitin (30 mcg)	-	+	-	-	+
Cloxacillin (5 mcg)	-	-	-	-	+
Ceftriaxone (30 mcg)	+	+	+	-	-
Vancomycin (30mcg)	-	-	-	-	+
Ceftazidime (30mcg)	+	+	+	+	-
Cefixime (5 mcg)	+	+	+	-	-
Cefepime (30 mcg)	+	+	+	+	-
Aztreonam (30 mcg)	+	+	+	+	-
Imipenem (10mcg)	+	+	+	+	+
Meropenem (10mcg)	+	+	+	+	+
Co-trimoxazole (1.25/23.75 mcg)	+	+	+	-	+
Gentamicin (10 mcg)	+	+	+	+	+
Netilmicin (30 mcg)	+	+	+	+	+
Doxycycline (30 mcg)	+	+	+	-	+
Tetracycline (30 mcg)	+	+	+	-	+
Linezolid (30mcg)	-	-	-	-	+
Erythromycin (5 mcg)	-	-	-	-	+
Azithromycin (15 mcg)	-	-	-	-	+
Chloramphenicol (30 mcg)	+	+	+	+	+
Colistin (10 mcg)	-	+	-	-	-
Piperacillin-Tazobactum (100/10mcg)	+	+	+	+	-

Quality control

Reference strains *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as a control reference strains for identifications and drug susceptibility testing. Negative control was done by randomly taking the prepared culture media and incubating 24 hours to see for any growth.

Data analysis

The information collected was reviewed and inconsistencies was investigated and clarified. Data were statistically described in terms of frequencies and percentages. All statistical calculations were done using computer program Statistical Package for the Social Science (SPSS, USA) version 20 for Microsoft Windows.

Results:

From the total 62 wound swabs, 49 (79%) were culture positive, 13 (21%) were negative (Figure I). Among 49 culture positive cases 34 (69.39%) were males and 15 (30.61%) were females.

Among the isolated organisms predominant bacteria was *Acinetobacter spp.* 20 (40.81%) followed by *Staphylococcus aureus* 10 (20.41%) *Escherichia coli* 07 (14.29%), *Pseudomonas aeruginosa* 07 (14.29%), *Klebsiella spp.* 04 (08.16%), and *Proteus spp.* 01 (02.04%). (Figure 2).

Antibiotic susceptibility patterns of bacterial isolates are elaborated in Table I and Table II.

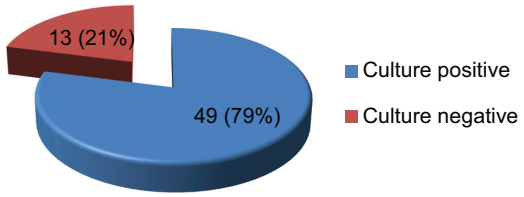

Fig.-1: *Pattern of bacterial growth among total samples (n=62)*

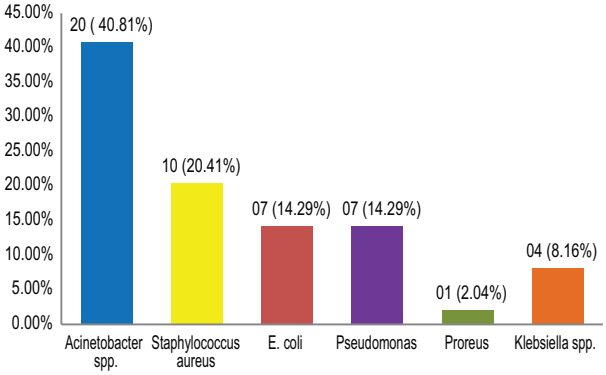

Fig.-2: *Rate of isolation of different bacteria (N=49)*

Table-I

Antibiotic resistance pattern of isolated Gram negative bacteria.

Antibiotic	Acinetobacter spp (20)	*E.coli* (07)	Pseudomonas spp (07)	Klebsiella (04)
Co-trimoxazole	17(85%)	04(57.14%)	***	02 (50%)
Gentamicin	15(75%)	02 (28.57%)	01 (14.28%)	04 (100%)
Ciprofloxacin	14(70%)	03(42.85%)	02 (28.57%)	04 (100%)
Levofloxacin	14(70%)	03(42.85%)	02 (28.57%)	04 (100%)
Cephradine	20(100%)	07 (100%)	***	04 (100%)
Cefoxitin	***	03(42.85%)	***	***
Cefixime	15(75%)	06 (85.71%)	***	04 (100%)
Ceftriaxone	16(80%)	06 (85.71%)	***	04 (100%)
Ceftazidime	10(50%)	03(42.85%)	03 (42.85%)	03 (75%)
Cefepime	07(35%)	02 (28.57%)	02 (28.57%)	04 (100%)
Aztreonam	10(50%)	05(71.42%)	01 (14.28%)	04 (100%)
Imipenem	03(15%)	00	01 (14.28%)	00
Meropenem	03(15%)	00	01 (14.28%)	00
Netilmicin	13(65%)	01 (14.28%)	02 (28.57%)	03 (75%)
Doxycycline	13(65%)	03(42.85%)	***	04 (100%)
Tetracycline	15 (75%)	03(42.85%)	***	04 (100%)
Chloramphenicol	14(70%)	01 (14.28%)	04 (57.14%)	03 (75%)
Amikacin	14(70%)	02 (28.57%)	03 (42.85%)	04 (100%)
Colistin	00	00	***	***
Piperacillin-Tazobactam	15 (75%)	03(42.85%)	00	03 (75%)

*** = Susceptibility not done.

Table II
Antibiotic resistance pattern of isolated Gram-positive bacteria.

Antibiotic	Staphylococcus Spp (10)
Amikacin	03 (30%)
Cephradine	00
Cefoxitin	00
Cloxacillin	00
Vancomycin	00
Gentamycin	04(40%)
Netilmycin	04(40%)
Doxycycline	02(20%)
Tetracycline	02(20%)
Chloramphenicol	01 (10%)
Linezolid	00
Imipenem	00
Meropenem	00
Erythromycin	09 (90%)
Azithromycin	09 (90%)
Cotrimoxazole	07 (70%)

Discussion:

Wound infection is a major concern among healthcare practitioners, not only in terms of increased trauma to the patient but also increase cost of effective management within the health care system.[14]

In the developing countries like Bangladesh, physicians prescribe antimicrobial more than the actual need, all kinds of antibiotics are easily available over the counter and anybody can buy drugs without physician's prescription are responsible for developing pool of resistant bacteria as well as negative results of culture.[15]

In spite of proper application of the basic principles of wound care a number of patients develop infections, needing proper identification of the organisms for appropriate management. A changing pattern of isolated organisms and their antimicrobial sensitivity varies from hospital to hospital and region to region is a usual feature.[16]

In this study, 79% was culture positive which was almost same (83.65%) to a study done in another private diagnostic centre in Dhaka.[17] In other studies, the recovery rate of microbial pathogens from wound swab were 68.8% in Ugand[18], 71% in Ethiopia[19] and 60.6% in Nepal.[20] The proportion of culture positivity was comparatively high at 90% in Tanzania[21] and 96% in India.[22] This showed that there are inter country variation may be due to differences in the infection control practices and differences in the population studied (co morbid illnesses, sex, age). In this study, 21% was culture negative, suggesting possibility of anaerobic organisms. Anaerobic culture was not done in this study.

The results of the present study showed that, in men culture positivity was high (69.39%) when compared with women (30.61%). The result was consistent with the study done by KC *et al.*[23] who reported high culture positivity in 78.18% men. This could be due to the higher involvement of males in physical outdoor works for earning livelihood as compared to females and more chances of accidents during the activities.

The preponderance of Gram negative bacteria in the current study was in agreement with findings from neighboring Tanzania and Ethiopia.[21], [24] This could be attributed to diverse habitat of Gram negative bacteria including inanimate surfaces in hospitals, multidrug resistant patterns and possible contamination from intestinal tract during surgery.

Acinetobacter species was the predominant isolates in this study that constituted 51.28% of the Gram negative isolates and 40.81% among the total bacterial isolates. Chim *et al.*[25] also found *Acinetobacter* spp. highly prevalent in Singapore and explained this situation by constant introduction of *Acinetobacter* spp. carried on human skin (endemic to tropical climate). Acinetobacter species are common contaminants in operating room air and fomites including medical equipments in hospitals.[26] Some studies reported *S. aureus* as the predominant isolate.[27,28] The possible reason for variation in the isolated organisms may be due to the differences in aseptic techniques followed, diverse geographical distribution of

causative agents and difference in the surgical procedures performed. When internal organs are resected through the abdomen, the causative agents included the normal Gram negative flora of the gut and in clean procedures, exogenous bacteria or skin colonizers is recovered.[22]

In the present study, Among the Acinetobacter spp. isolates, 70% was multidrug-resistant Acinetobacter or MDR Acinetobacter (resistance to three or more classes of antibiotics). In a study conducted by Kelper *et al.* [29], 29 (76.31%) *Acinetobacter spp.* was MDR. MDR *Acinetobacter* is an important nosocomial pathogen. It has the capacity to survive in dry environments, which increases the risk for nosocomial transmission.[30] Risk factors for colonization or infection with MDR Acinetobacter include length of hospital stay, surgery, wounds, and treatment with broad-spectrum antibiotics, parenteral nutrition, indwelling catheters, mechanical ventilation, and admission to an intensive care unit.[31]

The result of the present study showed resistance of the *Acinetobacter* species towards the piperacillin - tazobactam combination. Only 25% were sensitive to piperacillin - tazobactam combination. This is in accordance with studies from India and other countries, which also reported a high resistance rate of *A. speceies* isolates to piperacillin - tazobactam combination.[32, 33]

Almost all the antibiotics used in this study were found to be resistant to the Acinetobacter. However, the imipenem and meropenem were sensitive in 85% isolates. A recent study from India has shown 50% sensitivity of *Acinetobacter* species to carbapenems.[34] Carbapenems (imipenem and meropenem) are the mainstay of treatment for antimicrobial-resistant gram-negative infections, though carbapenem-resistant Acinetobacter is also increasing.[35]

In this study colistin was the only drug that showed 100% sensitivity against *Acinetobacter*. Van *et al.*[33] also reported 100% sensitivity to colistin. Additionally, Jaggi *et al.*[32] reported around 98.8% sensitivity and Rani *et al.*[34] reported 80%–90% sensitivity to Colistin. Correspondingly, Vakili *et al.*[36] from Iran reported 11.6% resistance to colistin. Colistin are the active antibiotics for the treatment of MDR Acinetobacter.[37] Colistin, is a narrow spectrum cationic lipopeptide rapidly bactericidal against gram-negative bacteria. Moreover, colistin administration alone is associated with significant nephrotoxicity and hetero-resistance in MDR *A. baumannii* clinical isolates. A meta-analysis suggested that colistin is probably as safe and efficacious as standard antibiotics for the treatment of drug-resistant *A. baumannii* infection.[38]

In the present study, E. coli showed highest susceptibility to imipenem and colistin (100%) followed by amikacin (71.42%); piperacillin - tazobactam (57.14%); ciprofloxacin (57. 14%), cefoxitin (57.14 %). A low susceptibility was observed with ceftriaxone (14.28%); aztreonam (28.57%). These findings are similar to the studies done by Kumar *et al*,[39] Mahmood *et al.*[40] Ranjan *et al.*[35] They reported that Gram negative isolates were found to be most susceptible to imipenem (100%) followed by piperacillin - tazobactam ((87.22%), third generation cephalosporin (31.11%), cefoxitin (31.11%), aztreonam (31.11%). The observation of ceftriaxone resistance pattern is suggestive of the fact that 85.72% *E. coli* isolates were extended spectrum beta-lactamase (ESBL) producers.

The *Klebsiella spp* isolates in this study were 100% resistant to ceftriaxone, aztreonam, cefepime, ciprofloxacin, gentamicin, amikacin. Imipenem also proved to be the most active antibiotic against the *Klebsiella spp*. isolates that was 100% sensitive. This report showed that the third and fourth generation cephalosporins were ineffective in the treatment of wound infection in this study. This was in agreement with the work of Iroha *et al.*[42] where 59.6% of the identified *Klebsiella spp* isolates were confirmed as ESBL producers.

In the present study, out of 07 *Pseudomonas spp.* isolates, 03 (42.85%) were found to be MDR isolates. Colistin and Piperacillin/ Tazobactam were absolutely effective (100%) against *Pseudomonas spp.* followed by imipenem (85.71%), meropenem (85.71%), gentamicin

(85.71%), levofloxacin (71.42%). Ceftazidime was found to be only 57.14% sensitive. A study done by Anbumani *et al.*[43] had shown susceptibility pattern for *Pseudomonas spp.* with imipenem 100%, piperacillin - tazobactum (87.71%), levofloxacin (85.71%), cefotaxime (71.42%). Similar study by Acharya[44] revealed that 62.16% MDR among *Pseudomonas spp.* isolates.

The study by Hani and Adnan[45] showed gentamicin effectiveness was 72%. But the study carried out by Falagas *et al.*[46] reported that the isolated organism was almost resistant to amikacin, gentamicin and other anti-pseudomonal antibiotics. The study conducted by Li *et al.*[47] showed that active efflux played role in the resistance to various non-â-lactam agents by *Pseudomonas spp.*

In our study, Staphylococcus aureus showed 100% sensitive to imipenem, meropenem, linezolid, vancomycin, cefoxitin, cloxacillin followed by 90% to chloramphenicol, 80% to tetracycline, 70% to amikacin, and 60% to gentamicin and less sensitivity were found in antibiotic like erythromycin 10%, azithromycin 10%, and cotrimoxazole 30%. This finding was in agreement with the work of Bess LJ, *et al.*[3] and Shamsuzzaman *et al.*[48] .Gautam R *et al*[49], reported that Staphylococci are 100% sensitive to vancomycin and to amikacin. Another study showed complete sensitivity to vancomycin, linezolid and amikacin[50] and low activities against co-trimoxazole, tetracycline and erythromycin.[51] ·The above findings are near about similar to our study findings. Unlike a reports in which MRSA was associated with wound infections[52] our findings revealed susceptibility in *S. aureus* isolates towards cloxacillin and cephalosporins.

Remarkable susceptibility of *Staphylococcus aureus* to vancomycin, linezolid, imipenem, meropenem may be due to lesser use of these antibiotics as a result of their less availability, cost and toxic effect[53]. Low activities of commonly used antibiotics such as cotrimoxazole, erythromycin, azithromycin may be due to increased consumption of a particular antibiotic develops resistance resulting from mutation at drug target sites.[54]. As a result, they have lost their efficacy in the treatment of wound infection.

There was variation in the antibiotic sensitivity rate of various organisms isolated in the present study when compared to different past studies. This may be due to the fact that sensitivity of organisms to antibiotics is variable and depends upon prevalence of strains, antibiotics use, and its resistance patterns in a particular area.

Conclusion

This study revealed that a variety of bacteria found in wound infection with high rate of resistance to most commonly used antibiotics to treat the infections. Therefore, timely investigation and monitoring antibiotic resistance pattern plays an important role in reduction of the incidence of wound infections.

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