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Pattern of Aerobic Bacterial Pathogens Causing Wound Infection at a Tertiary Teaching Hospital in Bangladesh with Special Reference to Methicillin Resistant *Staphylococcus aureus* and Extended Spectrum Beta Lactamases

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Abstract

Background: Wound infection gaining utmost importance day by day because of emergence of drug resistant bacteria. **Objective:** This study was carried out to see the pattern of aerobic bacterial pathogens along with MRSA and ESBL producing strains causing wound infection. **Methodology:** This cross sectional study was conducted from May 2010 to April 2011. Wound swabs were taken from the patients suffering from wound infections admitted in the different surgical wards of Rajshahi Medical College Hospital, Rajshahi, Bangladesh. The study place was the department of Microbiology of Rajshahi Medical College, Rajshahi and department of General Surgery, Orthopedic Surgery and Gynaecology & Obstetrics of Rajshahi Medical College Hospital, Rajshahi. Two wound samples were collected from each patient with, one for microscopy and other for culture. Gram staining and culture of collected wound swab were done for isolation and identification of MRSA and ESBL Bacteria. Antimicrobial susceptibility testing of the isolates were performed by using modified Kirby-Bauer technique using Mueller-Hinton agar and commercially available antimicrobial discs manufactured by Oxoid Diagnostic. **Results:** A total of 300 samples were collected. Culture yielded growth of 175(58.33%) cases, of which 138 (78.86%) were gram negative. From total 138 of gram negative bacteria 52(37.68%) were ESBL positive and they were distributed as *K. pneumoniae* 05(55.56%), *E. coli* 35(54.68%), *Pr. mirabilis* 05(20.83%) and *Ps. aeruginosa* 05(17.00%). All the ESBL producers were 100.0% resistant against ampicillin, cotrimoxazole, ceftriaxone and ceftazidime. Ciprofloxacin was 80.0 to 88.6%, gentamicin was 40.0 to 60.0%, aztreonam was 60.0 to 100.0% and netilmycin was 20.0 to 60% resistant. All the MRSA were 100.0% resistant against ampicillin, co-trimoxazole and cloxacillin. None showed resistance against vancomycin. **Conclusion:** Drug resistant ESBL producing bacteria and MRSA are quite high among the isolated pathogens with resistant to most of the antimicrobial agent. [*Bangladesh Journal of Infectious Diseases, June 2022;9(1):15-24*]

Keywords: Aerobic bacterial pathogens; wound infection; wound swab; MRSA; ESBL

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Introduction

Wound infection is a long continued problem throughout the world as well as in Bangladesh. It is a major cause of morbidity and mortality. It is gaining utmost importance day by day because of emergence of drug resistant bacteria. Human skin acts as an excellent mechanical barrier against microbial infection. Microbial infection usually occurs when there is a breach of continuity of skin or wound formation[1-2]. Bacteria are the commonest agents of the microbes and trauma is the commonest cause of wound. Trauma may be accidental like road traffic accident and burn and intentionally induced such as surgery and intravenous medical devices[3].

Clinically wound infection may be surgical, traumatic and burn and abscesses may occur as a consequence of wound infection. Among them surgical wound infections are most common. Many factors are responsible for wound infection such as integrity of the skin, virulence of microorganisms (bacteria and fungi), the host immune response. Wound infection is universal and the species of bacteria varies with geographical location, resident flora, clothing, site of wound and time between wound and management[4].

Prevalence of wound infection varies in different countries of world and a good number of wound infections are nosocomial. According to WHO the prevalence of wound infection is about 5-34% of all nosocomial infections. The nosocomial surgical site infection is 10% in the United Kingdom and 14-16% in USA[5]. The post-operative wound infection is 20% cases in India[6] and 31.37% cases in Bangladesh[7]. A wide range of bacteria are responsible for wound infection such as *Staphylococcus (S) aureus*, Coagulase Negative *Staphylococcus (CoNS), Enterococcus (En) faeacalis, Escherichia (Esch.) coli, Pseudomonas (Ps) aeruginosa, Enterobacter cloacae, Proteus (Pr) mirabilis, Klebsiella (K) pneumoniae* etc. are aerobic bacteria and *Clostridium (Cl) perfringens, Cl. tetani* etc. are anaerobic bacteria[8].

Surgical wound infection is a common post-operative complication and causes significant post-operative morbidity and mortality. Any purulent discharge from a closed surgical incision together with signs of inflammation within 30 days of an operation should be considered as surgical wound infection[9]. In 1992, the term surgical wound infection was replaced into surgical site infection by the Task Force for the surgical wound infection[10]. The bacteria responsible for surgical site infections are the patient's own normal flora or bacteria from the environment or hospital staffs.

Traumatic infections are other causes of wound infections. Most of the admissions in surgical emergency are due to traumatic injuries and infections causing 30.0 to 80.0% deaths[11]. Multiple factors responsible for traumatic infection including the mechanism of injury, site of injury & colonizing bacteria, number of bacteria in the wound and the time interval from injury to treatment[12]. The burn infection is another leading cause of morbidity and mortality. Burn is the damage of the skin caused by a variety of heat, chemicals, electricity, sunlight and nuclear radiation. Burn infection is problematic because it causes inflammation, bacterimia, septicemia, delay in healing, scar formation and multiple-organ dysfunction syndrome[13]. Bacteria and fungi are the common pathogens in burn infection. Both the bacteria and fungi form multi-species bio film on burn within 48 to 72 hours of injury and originated from the patient's own skin, gut and respiratory flora, contaminated health care environment and hospital staffs. Bacteria responsible for burn infection are methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faeacalis, Streptococcus pyogenes*, Coagulase Negative *Staphylococcus, Pseudomonas aeruginosa, Escherichia coli, Proteus* species[14]. and the fungi responsible are *Candida, Aspergillus, Fusobacterium* and so on[15]. Burn infection is quite common in third-world countries. In Bangladesh the rate of burn infection was 90% cases[16] and 75.0% cases[17], in Pakistan was 49.3% cases[18] and in Jordan it was 8.98% cases[15].

Skin abscess are also common problem. Usually occur after a minor wound or injury and bacterial infection. It may be caused by the obstruction of sweat glands and oil (Sebaceous) glands and inflammation of hair follicles. An untreated wound infection can also result in an abscess[19]. Abscesses can form in almost any part of the body, but the skin, under the skin and the teeth are the most common sites. Whatever the aetiological agent of wound infection is, the antimicrobial therapy is the choice of treatment. But the emergence of antimicrobial resistance has been coming up as the major therapeutic challenge. Methicillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta lactamase (ESBL) producing gram negative bacteria are the main concern among the drug resistant isolates. Methicillin-resistant *Staphylococcus aureus* is a major nosocomial pathogen worldwide and is potentially a great threat in medical therapy[20]. MRSA infections are increasingly reported from many countries world

wide[21]. Different studies showed that MRSA was 51.6% cases in India[22], 50.0% cases in Pakistan[23] and 62.5% cases, 70.2% cases and 83.3% cases[20,24-25] in Bangladesh. The molecular mechanism responsible for methicillin resistance is the presence of the staphylococcal cassette chromosome (SCC), a mobile element in the genome of *Staphylococcus aureus*. This SCC contains the *mecA* gene, which encodes the penicillin-binding protein 2a[26].

Methicillin-resistant *Staphylococcus aureus* usually isolated from a variety of clinical specimens but maximum isolation is from the chronic wound infections and other pyogenic infections[4]. To minimize this MRSA or other hospital acquired infections, wounds require aggressive treatment with appropriate topical and systemic antimicrobial agents[27]. The risk factors contribute to MRSA are excessive use of inappropriate antimicrobial agents, prolonged hospitalization specially in intensive care unit and intravascular catheterization. It has been found that many strains of MRSA exhibit resistance to both lactams and aminoglycosides. So it is necessary to select appropriate antimicrobial agents for the treatment of these infections[4]. Extended-spectrum β-lactamase producing organisms are also a major problem in the field of infectious disease management[28]. The mechanism of resistance of gram negative bacteria include the production of β-lactamase enzymes, alteration in the penicillin binding proteins, permeability of outer membrane and the combination of one or two of mentioned mechanisms[29]. ESBL are enzymes that mediate resistance to 3rd generation cephalosporin (ceftazidime, ceftriaxone, cefotaxime) and monobactams (aztreonam) but do not affect 2nd generation cephalosporins (cefoxitin, cefotetan) and carbapenems (meropenem or imipenem)[16]. Many ESBL-producing bacteria are also resistant to other antimicrobial namely aminoglycosides, trimethoprim, and quinolones.

Extended-spectrum β-latamases are the mutant, plasmid-mediated beta-lactamases derived from broad-spectrum beta-lactamases like TEM-1, TEM-2, SHV-1 which has an extended substrate specificity that permits hydrolysis of cephalosporins, penicillins and aztreonam[30]. There are also new members of ESBL family, including the CTX-M (cefotaxime M), OXA (oxacillinase) and unrelated β-lactamases[31]. These enzymes remain in the periplasmic space of gram negative bacteria and attack the antibiotics before it reaches its respective receptor site[32]. The β-lactams (penicillins, cephalosporins and carbapenems) are the most commonly used antimicrobial agents for the treatment of nosocomial infections[33], but persistent exposure of β-lactams lead to over production of mutated β-lactamases[34]. The ESBL producing strains are *Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli, Enterobacter species, Proteus mirabilis, Serratia* and *Pseudomonas* species[35].

These ESBL producing bacteria can cause both community and hospital acquired infections and are very difficult to treat with commonly used chemotherapeutic agents. ESBLs producing bacteria are a found in intensive care units, surgical wards and also in medicine wards[36]. The percentage of ESBLs producing bacteria varies from country to country. In India ESBLs in *Escherichia coli* was 58.1% cases and *Klebsiella* species was 43.7% cases; in Europe ESBLs in *Escherichia coli* was 5.4% and *Klebsiella* spp. was 23-25%; in Korea *E. coli* and *Klebsiella pneumoniae* was 4.87% cases, in Taiwan *Escherichia coli* and *Klebsiella pneumoniae* was 8.5% cases and in Hong Kong *Escherichia coli* and *Klebsiella pneumoniae* was 12.0% cases[16]. In Bangladesh ESBLs producing bacteria were 80.8% cases[16] and 47.27% cases[17]. The detection of ESBLs in the laboratory is difficult. Existence of extended-spectrum β-lactamases and their property for multidrug résistance will create serious problems in near future. Accurate laboratory detection is important to avoid treatment failure due to inappropriate antimicrobial therapy[33].

Although it has been noted in different studies that MRSA and ESBL producing bacteria are being isolated increasingly from wound infection cases but still most of the clinical microbiology laboratories in our country are not reporting them as routine. In most of the situations empirical third generation cephalosporin and penicillinase resistant penicillin is being used which have been found totally ineffective against ESBL producing gram negative isolates and MRSA respectively. To address the problem of infection control and formulation of antibiotic policy it is urgent to detect MRSA and ESBL producing bacteria from wound infection cases. The proposed study has designed to investigate the pattern of aerobic bacterial pathogens causing wound infection at RMCH along with their antibiogram. Attempt were also taken to determine the prevalence of MRSA and ESBLs producing isolates.

Methodology

Study Design and Population: This cross-sectional descriptive study was conducted from

May 2010 to April 2011. The wound swabs were collected from the patients suffering from wound infections admitted in the different surgical wards of Rajshahi Medical College Hospital.The study place was the department of Microbiology of Rajshahi Medical College, Rajshahi and department of General Surgery, Orthopedic Surgery and Gynaecology & Obstetrics of Rajshahi Medical College Hospital, Rajshahi. Wound swabs were taken from patients with wound infection. All kinds of wound swabs were taken. Inclusion criteria were patients of all age groups and both sexes with pus or discharge from the infection sites i.e. traumatic, burn, abscess and surgical site infections were included in the study. Exclusion criteria were patients of diabetic foot, burger's disease, decubitus ulcer and mycetoma were excluded.

Study Procedure: Standard microbiological method was used for collection of wound swab. Two wound samples were collected from each patient, one for microscopy and other for culture. Sterile cotton tipped swab was used to collect the samples with all necessary aseptic measures. Care was taken to avoid contact with surrounding skin. After collection, the swabs were put in the tubes and the tubes were capped with sterile cotton plugs properly. It was then labeled with patients' name and identification number and transferred to the Microbiology laboratory as early as possible

Laboratory Procedure: Microscopy was done in a uniform thin smear which was prepared with one of the two swabs. Then dried in air and fixed by flaming. Gram stain was done to all the fixed smears to see the morphology of bacteria and Gram reaction of any bacteria found, the morphology and arrangement (in case of coccus) were noted. Culture[37] of the specimens were inoculated in Blood agar, Nutrient agar, MacConkey's agar and Mannitol Salt agar. Before inoculation all the culture plates were dried in plate drier for 30 minutes. Then wound swab was applied to a small area of the plate, known as seed (A). A red hot sterilized inoculating wire loop was taken and then drawn from the seed in two or three parallel lines from the seed on to the fresh surface of the medium (B,B,B). This process was repeated as B, B, B to C,C,C then C,C, C to D,D,D and D,D,D to E,E,E. Care was taken to sterilize the inoculating loop and cool it by putting the loop into unseeded medium, between each sequence. At each plate the inoculum was derived from the most distal part of the immediately preceding strokes. Then the inoculated plates were incubated aerobically at 37^0C for 24 hours. Culture plates were examined in the next morning to see any bacterial growth. If culture plate showed the growth of bacteria then it was identified by their colony morphology, pigment production, haemolysis on blood agar plate, motility test, Gram staining and relevant biochemical tests[37]. The identified bacteria were sub cultured and processed for drug sensitivity test and preserved in nutrient agar slant for further use.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing[37] of the isolates were performed by using modified Kirby-Bauer technique using Mueller-Hinton agar and commercially available antimicrobial discs manufactured by Oxoid Diagnostic. *Staphylococcus aureus* and other gram positive bacteria were tested for sensitivity against ampicillin, cloxacillin, ciprofloxacin, gentamicin, cotrimoxazole and vancomycin. Gram negative bacteria other than *Pseudomonas* were tested for sensitivity against ampicillin, aztreonam, ceftriaxone, ciprofloxacin, gentamicin, imipenem, netilmycin and cotrimoxazole. *Pseudomonas* spp. were tested for sensitivity against Gentamicin, ciprofloxacin, aztreonam, ceftriaxone, ceftazidime, imipenem, netilmycin.

Quality Control: Quality control of Mueller Hinton media was performed by five % of prepared media was incubated without inoculation to check the sterility. Fresh batch of media prepared was tested for its ability to support the growth of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. Quality control of antimicrobial disc was done by a representative disc of each batch was tested with reference control bacterial strain viz. *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.

Procedure of Antimicrobial susceptibility test: Antimicrobial susceptibility test[38] was done by inoculum standardization where Three to five isolated colonies of similar appearance were taken with a sterile wire loop from a pure culture and put in a sterile test tube containing 2 ml sterile normal saline. The inoculum size was standardized by adjusting the turbidity with 0.5 McFarland's standard after adding normal saline drop by drop. Inoculation of identified bacteria and placement of disc[37] was done in the way where Mueller Hinton agar plates were used for drug sensitivity test. A zone of inhibition produced by test bacteria against each antimicrobial agent was categorized into sensitive (S) and resistant (R) was noted.

Detection of MRSA: Detection of MRSA of isolated *S. aureus* in the following way. All *Staphylococcus aureus* isolates were tested to detect MRSA using oxacillin (1 μg) disc. The inoculum

size was adjusted with 0.5 McFarland's standard and incubating at 35^0C for 24 hours. A zone of inhibition less than 10 mm or any discernable growth within zone of inhibition was indicative of methicillin resistance. On the other hand, a zone of inhibition equal to or more than 13mm were taken as sensitive. Oxacillin was used in place of methicillin to detect MRSA for its stability[42].

Detection of ESBL: Detection of ESBL[43] was done in the following way All Gram negative bacilli those showed resistance to extended spectrum third generation cephlosporin and monobactams were tested for detection of ESBL production by modified double disc test and phenotypic confirmatory test. Among third generation cephalosporins like Ceftriaxone and ceftazidime and aztreonam were used from monobactams. Modified double disc diffusion test[44] is the method where synergy between the discs of augmentin (amoxycillin 20 µg plus clavulanic acid 10 µg) and 3rd generation cephalosporins and monobactums were observed. The clavulanic acid in augmentin disc diffuses through the agar and inhibits the β-lactamase surrounding the 3rd generation cephalosporins and monbactum discs. Muller-Hinton agar plates were prepared and inoculated with standardized inoculum corresponding to 0.5 McFarland's standard with the help of sterile cotton swab. Augmentin disc was placed in the center of the plate. Third generation cephalosporins (ceftazidime, ceftriaxone and cefotaxime) and monobactum (aztreonam) were placed 20-30 mm away from the augmentin disc. The plates were incubated overnight at 37^0C. Extended spectrum beta lactamase production was considered positive when the zones of inhibition around the test antimicrobial discs were enhanced towards the augmentin disc.

Phenotypic Confirmatory Test of ESBL: Phenotypic confirmatory test[45] is a modified double disc diffusion test. Positive bacteria were tested by phenotypic confirmatory test. The confirmation of ESBLs producing isolates were done by inhibitor potentiated diffusion test according to CLSI recommendation. Third generation cephalosporins i.e. cefotaxime (30µg) and ceftazidime (30µg) disc alone and in combination with clavulanic acid (10µg) were used for this test. Combinations of ceftazidime and cefotaxime disc with clavulanic acid were prepared in the laboratory by using stock solution of clavulanic acid at 1000µg/ml. From this stock solution, 10µl of clavulanic acid solution was added to cefotaxime and ceftazidime discs one hour before applying to the plates inoculated with the test orgamisms. Ceftazidime and cefotaxime discs without clavulanic acid were placed on one side of inoculated plate and ceftazidime, cefotaxime disc combined with clavulanic acid were placed on other side of plates. Then the plates were incubated at 37^0C overnight. After overnight incubation zone of inhibition was measured. It was observed whether there was increase in the diameter of zone of inhibition for cefotaxime and ceftazidime in combination with clavulanic acid compared to its zone of inhibition for cefotaxime and ceftazidime tested alone. Interpretation of phenotypic confirmatory test [42] in the following way Five mm or >5mm increase in a zone of inhibition for cefotaxime and ceftazidime in combination with clavulanic acid than the zone of inhibition of cefotaxime and ceftazidime when tested alone were confirmed as an ESBL producing organism.

Statistical Analysis: Statistical analysis was performed by Statistical Package for Social Sciences (SPSS) version 22.0. Qualitative data were expressed as frequency and percent. The quantitative data were expressed as mean with standard deviation.

Ethical Clearance: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local ethics committee.

Results

A total of 300 wound swabs were collected from wound infection cases and were cultured in different bacteriological culture media. Among them 201(67%) from surgical site infections, 47(15.67%) from traumatic wound infections, 25(8.33%) from burn and 27(9%) from abscesses and culture yielded growth of 175(58.33%) cases and they are distributed as 118 (58.71%), 29(61.70%), 16(64%) and 12(44.44%) respectively (Table 1).

Table 1: Distribution of Clinical Samples and Their Growth in Wound Infection Cases (n=300)

Clinical Samples	Number of Samples	Culture Positive
Surgical site Infec.	201(67.0%)	118(58.71%)
Traumatic Wound	47(15.67%)	29(61.70%)
Burn	25(8.33%)	16(64.0%)
Abscess	27(9.0%)	12(44.4%)
Total	**300(100.0%)**	**175(58.3%)**

There were 7 age groups like 0 to 10 years, 11 to 20 years, 21 to 30 years, 31 to 40 years, 41 to 50 years, 51 to 60 years and above 60 years. Male were 103 (34.33%) and female were 197(65.67%) respectively. Male-Female ratio was 1:1.9 (Table 2).

Table 2: Age and Sex Distribution of Wound Infection Cases (n=300)

Age Group	Male	Female
0 to 10 Years	5(1.7%)	3(1.0%)
11 to 20 Years	13(4.3%)	34(11.3%)
21 to 30 Years	28(9.3%)	104(34.7%)
31 to 40 Years	22(7.3%)	27(9.0%)
41 to 50 Years	11(3.7%)	15(5.0%)
51 to 60 Years	10(3.3%)	8(2.7%)
>60 Years	14(4.7%)	6(2.0%)
Total	**103(34.3%)**	**197(65.7%)**

The distribution of ESBL and MRSA strains from different clinical samples of wound infection cases was recorded. From total 138 of gram negative bacteria 52(37.68%) were ESBL positive. Among the total 138 gram negative isolates 92(66.64%) were from surgical site infections (SSIs), 24(17.4%) from traumatic wounds, 13(9.42%) from burn and 9(6.5%) from abscesses. Among 52 ESBL strains 36(39.13%) were from 92 isolates of SSIs, 6(25%) were from 24 isolates of traumatic wound, 4(30.77%) were from 13 isolates of burn and 6(66.67%) were from 9 isolates of abscesses. Among 30 isolated *S. aureus* 20(66.67%) were MRSA. Of the total 30 *S. aureus* 23(76.67%) were from SSIs, 3(10%) from traumatic wound 3(10%) from burn and 1(3.33%) were from abscess. From SSIs 15(65.23% from traumatic wound were MRSA among 23 isolates, 2(66.67%) were MRSA among 3 isolates, from burn 3(100%) were MRSA among 3 isolates (Table 3).

Table 3: Distribution of ESBL and MRSA Strains from Different Clinical Samples

Clinical samples	Gram negative bacteria	ESBL strains	Gram positive bacteria	MRSA strains
Surgical infection	92(66.6%)	36(39.1%)	23(76.7%)	15(65.2%)
Traumatic wound	24(17.4%)	6(25.0%)	3(10.0%)	2(66.7%)
Burn	13(9.4%)	4(30.8%)	3(10.0%)	3(100.0%)
Abscess	09(6.5%)	6(66.7%)	1(3.3%)	0(0.0%)
Total	138(100.0%)	52(37.7%)	30(100.0%)	20(66.7%)

Among 138 identified Gram negative bacteria 52(37.68%) were ESBL producers of them *Klebsiella* spp. was 5(55.56%), *E. coli* was 35(54.68%), *Proteus spp.* was 5(20.83%) and *Ps. aeruginosa* was 7(17.00%) (Table 4).

The antimicrobial resistance pattern among the ESBL producing gram negative bacteria. All the ESBL producers were 100.0% resistant against ampicillin, co-trimoxazole, ceftriaxone and ceftazidime. Ciprofloxacin was 80 to 88.6%, gentamicin was 40.0 to 60.0%, aztreonam was 60.0 to 100.0% and netilmycin was 20.0 to 60.0% resistant. None showed resistance for imipenem (Table 5).

Table 4: Distribution of ESBL Strains among Identified Gram Negative Bacteria

Identified Gram Negative Bacteria	ESBL strains	Total
Klebsiella pneumoniae	5(55.6%)	9
Escherichia coli	35(54.7%)	64
Proteus mirabilis	5(20.8%)	24
Pseudomonas aeruginosa	7(17.0%)	41
Total	**52(37.7%)**	**138**

Table 5: Antimicrobial Resistance Pattern among the ESBL producing Gram Negative Bacteria (n=52)

Antimicrobial agents	*E. coli* (n=35)	*P. aeruginosa* (n=7)	*Pr. mirabilis* (n=5)	*K. pneumoniae* (n=5)
Ampicillin (10µgm)	35(100.0%)	Not used	5(100.0%)	5(100.0%)
Cotrimoxazole(25µgm)	35(100.0%)	Not used	5(100.0%)	5(100.0%)
Ciprofloxacin(10µgm)	31(88.6%)	6(85.7%)	4(80.0%)	4(80.0%)

Antimicrobial agents	***E. coli* (n=35)**	***P. aeruginosa* (n=7)**	***Pr. mirabilis* (n=5)**	***K. pneumoniae* (n=5)**
Gentamicin(10µgm)	21(60.0%)	4(57.1%)	3(60.0%)	2(40.0%)
Ceftriaxone(25µgm)	35(100.0%)	7(100.0%)	5(100.0%)	5(100.0%)
Ceftazidime(25 µgm)	35(100.0%)	7(100.0%)	5(100.0%)	5(100.0%)
Aztreonam(10 µgm)	29(82.8%)	7(100.0%)	3(60.0%)	4(80.0%)
Imipenem(10 (gm)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Netilmycin(10µgm)	13(37.1%)	3(42.85)	1(20.0%)	3(60.0%)

All the MRSA were 100% resistant against ampicillin, co-trimoxazole and cloxacillin. ciprofloxacin was 75% and gentamicin was 55% resistant. None showed resistance against vancomycin (Table 6).

Table 6: Antimicrobial resistance pattern of MRSA (n=20)

Antimicrobial agents	**MRSA strain**
Ampicillin (10 µgm)	20(100.0%)
Cotrimoxazole (25µgm)	20(100.0%)
Cloxacillin (5 µgm)	20(100.0%)
Gentmicin (10 µgm)	11(55.0%)
Ciprofloxacin (10 µgm)	15(75.0%)
Vancomycin (30 µgm)	0(0.0%)

Discussion

Wound infection is a major cause of mortality and morbidity[12]. It is one of the most common clinical problem that a surgeon faces in their daily practice. This problem has been existing from ancient time. In 14-17 AD Roman physician Correlius Celsus described the four principal signs of inflammation and described the treatment by using antiseptic solutions[8]. Till now it is a great threat for the health services in developing countries.

Wound can be caused by various types of injury but all types are not equally infected. Various factors are responsible for wound infection and the severity also varied person to person. Surgical wound infection cases are higher in contaminated cases than in clean cases[45]. Wound caused by accidents such as road traffic accident, gunshot, fall from height etc. can be contaminated by both anaerobic and aerobic bacteria during injury. But anaerobic contaminations are more than aerobic contamination[46]. The burn wounds are also likely to be colonized and infected by colonizing g aerobic bacteria which influences the risk and the degree of infection[15].

A total of 300 wound swabs were collected from different wound infections and cultured on appropriate bacteriological culture media and yielded 175 (58.3%) growth of bacteria. In surgical site infection, 118 (58.71%) bacteria were isolated. Our study is nearly similar with the study of Haque and Salam[47] in Bangladesh and Zhang[48] in China where they showed 55.5% and 47.9% growth. However, this study is different from other countries, such as 5.0% in USA[49] and 11.3% in India[6]. This dissimilarity may be due to the fact that bacterial predominance varies in different geographical locations, climate, food habit, improved hospital environment, proper aseptic measures, proper nutrition and medical education.

In traumatic wounds, 29(61.7%) bacteria were isolated. This study is nearly similar with the findings of Jahan et al[25] in Bangladesh they showed 68.0% and Jodie et al[50] in USA they showed 69.0% infection. Among burn cases, 16 (64%) bacteria were isolated. Our study is dissimilar with the studies of Ahmand et al[18] and Alim[16] they showed isolated bacteria were 49.3% and 90.0% respectively. These dissimilarities may be due to environmental contamination and presence of microbial flora on patient, attendants and medical personnel.

Among them 103(34.33%) were male and 197(65.67%) were female. The female is predominant due to a good number of cases were taken from Obstetrics and Gynae department. Among them 132 (44%) were from 21 to 30 years and 49(16.33%) were from 31 to 40 years of ages. This study is similar with Kowli et al[51] in India. The wound infection rate was higher in the elderly age groups which was 83.33% in 51 to 60 years and 80.0% in more than 60 years. This study is similar with the Haque[17], who reported infection cases were 84.62% in the patient of age more than 60 years. This higher infection cases in elderly patients may be due to the presence of co-morbidity, malignancy, immunosuppression and senile disorders.

The isolated gram negative bacteria were further tested to detect extended-spectrum beta lactamase (ESBL) producing strains. In this study ESBL

producers are 52(37.68%). This is nearly similar with Jabeen[28] in Pakistan, Haque[17] and Alim[16] in Bangladesh. They showed 30.0%, 46.67% and 41.39% are ESBL producers. But different findings were reported by Jones et al[52] in Vietnam (14.7%) and Jamal et al[53] in Kuwait (14.4%). This difference may be due to the fact that it is difficult to detect ESBL producers and its distribution varies between various geographical locations and hospitals. In this study out of 52(37.68%) ESBL producers, 66.7%, 39.1%, 30.8% and 25.0% are detected from isolates recover from abscess, surgical site infection, burn and traumatic wound. This finding is nearly similar with Haque[17] and Alim[16] in Bangladesh and observations were 47.3% and 33.2% and were detected from surgical site infection and burn respectively.

Methicillin resistant *S. aureus* (MRSA) is another therapeutic challenge like ESBL producing bacteria. Nowadays MRSA is increasing in hospitals of all sizes, health care centers, different population groups and various communities all over the world[54]. Out of 30 *S. aureus* 20(66.67%) isolates are MRSA. Among them 15(65.22%) are isolated from surgical site infection, 2(66.67%) from traumatic wound, 3((100%) from burn, but none from abscess. Our study is nearly similar with the Khan et al[24] and Afroz[20] in Bangladesh and their finding was 62.5% and 70.2% respectively. But different finding was reported by Jahan et al[25] in Bangladesh (88.3%) and Vidhani et al[22] in India (51.6%). This difference may be due to MRSA infection is variable from different hospitals, geographical locations and countries depending on antibiotic policy.

The distribution of ESBL producing gram negative bacteria were recorded. Out of 52 ESBL producing strains *E. coli* is 35(54.68%), *P. aeruginosa* is 7(17%), *Pr. mirabilis* is 5(20.83%) and *K. pneumoniae* is 5(55.56%). Both ESBL and MRSA strains are multi drug resistant. In this study all the ESBL strains are sent percent resistant to ampicillin, cotrimoxazole, ceftriaxone and ceftazidime. Nearly 80.0% resistance was observed against ciprofloxacin and aztreonam and relatively lower in netilmycin and gentamicin. But all are sensitive for imipenem. In other word imipenem, netilmycin and gentamicin are effective against ESBLs. Our study is nearly similar with Islam[40] in Bangladesh and he showed resistance against ampicillin, ceftriaxone,ceftazidime, cotrimoxazole, gentamicin, netilmycin was 100.0%, 100.0% 71.0 to 87.5%, 71.0%, 66.0%. But imipenem was 100.0% sensitive. This increased resistance may be due to extensive use of 3rd generation cephalosporin and other beta lactum drugs. In this study most of the patients were treated with 3rd generation cephalosporin and they also showed resistance against other antibiotics which indicate that ESBL producers are multidrug resistant. This multidrug resistance is that they contain resistance genes along with ESBL producing genes[47]. Imipenem is highly sensitive as it is newly marketed and not use routinely.

The isolated MRSA strains are sent percent resistant to ampicillin, co-trimoxazole, cloxacillin and 75.0% to ciprofloxacin. But all are sensitive to vancomycin. This finding is consistent with Afroz[20] in Bangladesh and Anuparba[4] in India. Vancomycin acts on cell wall but do not contain beta-lactum ring and is not inactivated by beta lactamases.

Conclusion

In this study, it was observed that a variety of bacteria are responsible for wound infection and among them a large number of bacteria are drug resistant. Surgical site infection is most common followed by traumatic wound, burn and abscesses. Wound infection cause prolonged hospital stay, increased cost of treatment and increased chance of infection with MRSA and ESBL. It is very important to identify the causative agent and give appropriate antimicrobial therapy by doing culture and sensitivity testing of all cases. It is well documented that a variety of aerobic and anaerobic bacteria are responsible for wound infection. It can occur at any age and both sexes. In the past it was thought that *S. aureus* is the commonest causative bacteria, but nowadays many gram positive and negative bacteria have responsible for it and a large number of them are drug resistant. So treatment of wound infection is gradually becoming difficult. It is well established that MRSA and ESBL producing strains are challenging in wound management. It is necessary to perform sensitivity test prior to start antimicrobial therapy for proper wound management and routine screening test for MRSA and ESBL should be practiced to detect the carriers and treat them adequately.

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Conflict of Interest
The authors have no conflicts of interest to disclose

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Contribution to authors:
Aftab S conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Tarik

MM, Kadir ML, Yusuf MA contributed to the analysis of the data, interpretation of the results and critically reviewing the manuscript. Yusuf A involved in the manuscript review and editing. All authors read and approved the final manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. The written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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