

Antibiotic Susceptibility Pattern of Aerobic and Anaerobic Bacteria Isolated From Surgical Site Infection of Hospitalized Patients

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Background: Surgical Site Infections (SSIs) are infections of incision or deep tissue at operation sites. These infections prolong hospitalization, delay wound healing, and increase the overall cost and morbidity.

Objectives: This study aimed to investigate anaerobic and aerobic bacteria prevalence in surgical site infections and determinate antibiotic susceptibility pattern in these isolates.

Materials and Methods: One hundred SSIs specimens were obtained by needle aspiration from purulent material in depth of infected site. These specimens were cultured and incubated in both aerobic and anaerobic condition. For detection of antibiotic susceptibility pattern in aerobic and anaerobic bacteria, we used disk diffusion, agar dilution, and E-test methods.

Results: A total of 194 bacterial strains were isolated from 100 samples of surgical sites. Predominant aerobic and facultative anaerobic bacteria isolated from these specimens were the members of *Enterobacteriaceae* family (66, 34.03%) followed by *Pseudomonas aeruginosa* (26, 13.4%), *Staphylococcus aureus* (24, 12.37%), *Acinetobacter* spp. (18, 9.28%), *Enterococcus* spp. (16, 8.24%), coagulase negative *Staphylococcus* spp. (14, 7.22%) and nonhemolytic streptococci (2, 1.03%). *Bacteroides fragilis* (26, 13.4%), and *Clostridium perfringens* (2, 1.03%) were isolated as anaerobic bacteria. The most resistant bacteria among anaerobic isolates were *B. fragilis*. All Gram-positive isolates were susceptible to vancomycin and linezolid while most of *Enterobacteriaceae* showed sensitivity to imipenem.

Conclusions: Most SSIs specimens were polymicrobial and predominant anaerobic isolate was *B. fragilis*. Isolated aerobic and anaerobic strains showed high level of resistance to antibiotics.

Keywords: Surgical Site Infections; Antibiotic Susceptibility Pattern; Polymicrobial Infection; Minimal Inhibitory Concentration

1. Background

Surgical Site Infections (SSIs) are defined as infections afflicting either the incision or deep tissue at the operation site. These infections occur within one year of a surgical procedure with an implant and within 30 days without any left implant. They are further categorized in terms of anatomic location: superficial infections afflict only skin or subcutaneous tissue around the incision; deep infections afflict deep soft tissue such as fascia and muscles; organ space infections involve any part of the body, excluding the skin incision, fascia or muscle layers, that is opened or manipulated during the operative procedure (1, 2). In spite of advance in infection control methods such as sterilization method, use of antibiotics for prophylaxis and surgical technique, SSIs have remained as a postoperative complication (3). These infections can increase the costs and duration of hospitalization. More-

over they can cause higher risk of morbidity and lower life quality in surgical patients (4, 5).

The rate of SSI lies between 15% and 20% depending mainly on the type of surgical procedure and the wound classification. SSI rates were 4.88% in clean cases, 8.39% in clean-contaminated cases, and 20.45% in dirty cases. SSI rates were highest in gastrointestinal surgeries as 80% of these infected cases were mostly dirty cases (6, 7). Several factors affect the occurrence of SSIs. These factors are categorized to patient factors, preoperative factors, intraoperative factors, and postoperative factors (2, 8). SSIs are often polymicrobial and microbiology of these infections is seldom static, including aerobes and anaerobes organism. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, the members of *Enterobacteriaceae* family, *Streptococcus* spp., *Enterococcus* spp., and *Acinetobacter* spp. are the predomi-

nant aerobic organisms that were reported in these infections (9).

The development of selective media and precise laboratory protocols for the recovery and identification of anaerobic bacteria have greatly enhanced our knowledge of their clinical importance. Most common anaerobe organisms that were reported from SSIs are *Fusobacterium* spp. (10, 11). In one microbiology study accomplished by Munez et al. *Escherichia coli* (8%), *Enterococcus* spp. (15%), *Streptococcus* spp. (8%), *P. aeruginosa* (7%), *S. aureus* (7%), *Bacteroides* spp. (3.6%), and *Clostridium* spp. (9%) were isolated from SSIs (12). In another study by Saini et al. the isolated bacteria from these infections were *E. coli*, *S. aureus*, *Klebsiella* spp., *P. aeruginosa*, *Bacteroides fragilis*, and *Peptostreptococcus* spp. (9). In one study performed by Wolcott et al. molecular method was applied for the detection of bacterial prevalence in surgical site infections. Results reported by these researchers indicated the bacteroides group as the most isolated anaerobic bacteria amongst anaerobes isolated from specimens (10). In 2006 Akhi et al. isolated *S. aureus* (37.5%) and *Enterobacteriaceae* spp. (32.5%) as the predominant bacteria from postoperative infections in different surgery wards (13).

2. Objectives

Owing to low antibiotic susceptibility in aerobic and anaerobic bacteria and presence of these bacteria in SSIs, both empirical therapy and antimicrobial prophylaxis administration may not be appropriate for these infections. Therefore, microbiology laboratory has a vital role in identification and determination of antibiotic susceptibility pattern in these infections. So, the aim of this study was to investigate anaerobic and aerobic bacteria profile in surgical site infections and determination of antibiotic susceptibility pattern in these isolates.

3. Materials and Methods

3.1. Hospital Setting

Imam Reza hospital with 520 beds in 26 clinical wards, including nephrology, gastroenterology, pulmonology, endocrine and rheumatology, neurology, infectious disease, ICU of neurology, ICU of neurology surgery, ICU of general surgery and of pulmonary, is situated in Tabriz City. This hospital provides services for nearly 2000 patients every month. Hospital environment cleaning practice is performed by special team regulatory with water, detergent and disinfectant. The most common way of staff hand hygiene is hand washing with soap and water and big spray usually are applied for tables cleaning.

3.2. Collection of Specimens

Between October 2012 and July 2013, 100 SSIs specimens (kind of infections and anatomic location were not considered in sampling) obtained from selected patients,

hospitalized in Imam Reza Hospital. Some of the patients were undergoing treatment with antibiotic drugs such as metronidazole, vancomycin, clindamycin, imipenem, ceftriaxone, and other cephalosporins. All collected specimens were processed for the detection of anaerobe and aerobic bacteria in medical microbiology laboratory of medicine faculty. For sampling, infected site was first scrubbed with povidone-iodine and culture specimens were obtained by in depth needle aspiration of material in the infected site (9). First of all a drop of aspiration was introduced to thioglycolate broth medium and then syringe was immediately sealed (9, 14). Specimens were transported to laboratory within 20 minutes and generally inoculated within 1 hour after collection.

3.3. Microbial Investigation

A Gram-stain smear was used for cytology investigation and detection of bacterial presence in specimens. For the isolation of aerobic organisms, specimens were plated onto chocolate, sheep blood (5%) (Liofilchem) phenylethyl alcohol (PEA) (Hi Meia, India), and MacConkey agar (Liofilchem, Italy) plate. The plates were incubated at 37°C under 10% CO₂ and examined at 24 hours and 48 hours later. Pre-reduced vitamin K enriched brucella blood agar; kanamycin-vancomycin laked blood agar (KVLB, Basal Medium is Brucella agar; Fluka Chemie AG CH-9471 Buchs, Switzerland), bacteroides bile esculin (BBE, Himedia Laboratories Pvt. Ltd, India) and phenylethyl alcohol (PEA) agar were inoculated for isolation of anaerobic organisms. The plate media were incubated under 80% N₂, 10% CO₂, 10% H₂, and 0% O₂ in anaerobic jar by using Anoxomat (MART microbiology B.V. The Netherlands) and these plates examined at 48, 72, and 96 hours. The primary inoculated thioglycolate broth (Merck Co., Germany) was incubated for 10 days and subcultured in 2 series of plates in the same way mentioned above. For enrichment and isolation of *C. perfringens*, a drop of syringe specimen was introduced into cooked meat broth media (Que Lab Inc) and incubated at 45°C for 4 - 6 hours. Thereafter, one loop of this incubated media was subcultured in sheep blood agar plate and incubated under anaerobic condition and examined after 24 and 48 hours. All isolated anaerobes were identified after conducting anaerobic tolerance test using biochemical tests such as catalase production, indole, and sugar fermentation (sucrose, arabinose, xylose, and rhamnose) as well as MID8 (Mast Identification 8, according to manufacturer company's instructions) (14, 15).

3.4. Antibiotic Susceptibility Testing

For investigation of antibiotic susceptibility pattern in aerobic bacteria that isolated from these infections, we performed antibiotic susceptibility test by Kirby-Bauer method (disk diffusion test) in Muller-Hinton agar (Liofilchem Ltd, Italy) using CLSI guideline (16). Imipenem (10 µg), gentamicin (10 µg), amoxicillin-clavulanic acid (20.10

µg), ciprofloxacin (5 µg), ceftazidime (30 µg), tetracycline (30 µg), piperacillin-tazobactam (100.10 µg), chloramphenicol (30 µg), and colistin (10 µg) were used for testing Gram-negative bacilli and erythromycin (15 µg), vancomycin (30 µg), clindamycin (2 µg), gentamicin (10 µg), ceftazidime (30 µg), oxacillin (1 µg), piperacillin-tazobactam (100.10 µg), linezolid (30 µg), chloramphenicol (30 µg), amoxicillin-clavulanic acid (20.10 µg), and rifampin (5 µg) were used for testing Gram-positive bacteria isolated from these infections (All disks were provided from Mast Ltd.).

For antimicrobial drug susceptibility assay in Gram-negative anaerobic bacteria isolated from these infections, the Minimum Inhibitory Concentration (MIC) of imipenem, chloramphenicol, metronidazole, clindamycin, ceftazidime, and penicillin G (Sigma chemical Co. USA) was determined by the agar dilution method. MIC of penicillin, metronidazole, clindamycin, ceftazidime for Gram positive anaerobic bacteria were determined by Etest strip (AB biomérieux, Sweden) according to CLSI guideline for anaerobic susceptibility testing (17).

4. Results

In this study, 194 bacteria (166 (85.57%) aerobic and 28

(14.43%) anaerobic) were isolated from 100 SSI specimens obtained from 42 female and 58 male patients who had undergone surgery. The patients' ages ranged from 14 to 85 years. The results showed 82% polymicrobial nature of the surgical infections, which only 28% were mixed anaerobic-aerobic. Predominant aerobic bacteria isolated from these infections were members of *Enterobacteriaceae* family (66, 34.03%), including 24 (12.37%) *E. coli*, 20 (10.31%) *Klebsiella* spp. 12 (6.2%) *Enterobacter* spp. 4 (2.06%) *Serratia* spp. 4 (2.06%) *Morganella* spp. and 2 (1.03%) *Citrobacter* spp. followed by *P. aeruginosa* (26, 13.4%), *S. aureus* (24, 12.37%), *Acinetobacter* spp. (18, 9.28%), *Enterococcus* spp. (16, 8.24%), coagulase negative *Staphylococcus* spp. (14, 7.22%) and nonhemolytic *Streptococci* (2, 1.03%).

Predominant anaerobic bacteria were *B. fragilis* group (26, 13.4%) followed by *Clostridium perfringens* (2, 1.03%). In our study, 4 specimens were negative culture (96% positive), one bacterium was isolated from 14 specimens, 2 bacteria from 66 specimens and 3 bacteria from 16 specimens. All anaerobic isolated bacteria were mixed with aerobic and facultative anaerobic bacteria. Antibiotic susceptibility pattern of aerobic and anaerobic isolates are presented in Tables 1 and 2.

Table 1. Antimicrobial Susceptibility of Bacteria Isolated From Aerobic Cultures^a

Bacterial Strain	<i>Staphylococcus aureus</i> ^b	<i>Enterococcus</i> spp.	Coagulase-Negative <i>Staphylococci</i>	<i>Streptococcus</i> spp. Viridians group	<i>E. coli</i>	<i>Klebsiella</i> spp.	Other <i>Enterobacteriaceae</i>	<i>p. aeruginosa</i>	<i>Acinetobacter</i> spp.
VAN (30 µg)	100	100	100	100	NT	NT	NT	NT	NT
LZD (30 µg)	100	100	100	100	NT	NT	NT	NT	NT
OX (1 µg)	0	NT	0	NT	NT	NT	NT	NT	NT
C (30 µg)	8.3	25	28.6	0	41.7	20	45.5	NT	NT
CD (2 µg)	16.7	NT	42.9	0	NT	NT	NT	NT	NT
CIP (5 µg)	41.7	100	42.9	NT	33.3	10	36.4	53.9	11.1
T (30 µg)	16.7	50	14.3	0	33.3	30	27.3	NT	0
E (15 µg)	33.3	37.5	28.6	100	NT	NT	NT	NT	NT
GM (10 µg)	34.3	NT	0	NT	58.3	40	63.7	30.8	55.5
FOX (30 µg)	16.7	NT	14.3	100	50	20	54.6	NT	NT
PTZ (110 µg)	33.3	NT	42.9	NT	46.7	20	45.4	23.1	11.1
AUG (30 µg)	25	NT	14.3	NT	25	10	36.4	NT	NT
AP (10 µg)	NT	0	NT	NT	NT	NT	NT	NT	NT
RP (5)	41.7	25	42.9	NT	NT	NT	NT	NT	NT
IMI (10 µg)	NT	NT	NT	NT	100	100	100	46.1	22.2
CTX (30 µg)	NT	NT	NT	NT	25	30	36.4	NT	0
CO (10 µg)	NT	NT	NT	NT	NT	NT	NT	100	NT

^a Abbreviations: AP, Ampicillin; AUG, Amoxicillin-clavulanic acid; C, Chloramphenicol; CD, Clindamycin; CIP, Ciprofloxacin; CO, Colistin; CTX, Cefotaxime; E, Erythromycin; FOX, Ceftazidime; GM, Gentamicin; IMI, Imipenem; LZD, Linezolid; NT, Not Tested; OX, Oxacillin; PTZ, Piperacillin-tazobactam; RP, Rifampin; S, Susceptible; T, Tetracycline; VAN, Vancomycin.

^b Data are shown as S (%).

Table 2. Antimicrobial Susceptibility of Bacteria Isolated From Anaerobic Culture^a

Agents	Penicillin			Ceftazidime			Chloramphenicol			Clindamycin			Metronidazole			Imipenem		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Bacterial Strains MCI (µg/mL)	≤ 0.5	1	≥ 2	≤ 16	32	≥ 64	≤ 8	16	≥ 32	≤ 2	4	≥ 8	≤ 8	16	≥ 32	≤ 4	8	≥ 16
<i>B. fragilis</i> group	0	0	100	61.5	0	38.5	69.2	0	30.8	53.8	7.7	38.5	69.2	0	30.8	92.3	0	7.6
<i>C. perfringens</i>	100	0	0	100	0	0	NT	NT	NT	100	0	0	100	0	0	NT	NT	NT

^a I, Intermediate; MIC, Minimal Inhibitory Concentration; NT, Not Tested; S, Susceptible; R, Resistant.

5. Discussion

One common form of nosocomial infections are SSIs (4). Surgical site infections, and wounds with devitalized tissues are largely polymicrobial, and the role of both aerobic and anaerobic bacteria in the pathogenesis of these infections is well recognized (9). Microbial synergy may increase the net pathogenic effect and hence the severity of infection in several ways: 1) oxygen consumption by aerobic bacteria induces tissue hypoxia and a lowering of the redox potential, which favors the growth of anaerobic bacteria; 2) specific nutrients produced by one bacterium may encourage the growth of fastidious and potentially pathogenic cohabiting microorganisms; and 3) some anaerobes are able to impair host immune cell function and thus provide a competitive advantage for themselves as well as for other cohabiting microorganisms (11). Although the commonest bacterial strains (*Enterobacteriaceae*) that were isolated from specimens in this study are similar to the findings of other studies carried out by different researches (9, 12, 18), studies carried out by Giacometti et al. (19) and Mahesh (20) reported Gram-positive cocci, especially *S. aureus* as predominant bacterial isolate in SSIs (21). Surgical sites and the kind of operation could be the reason for such differences. In our study, most of the infected patients had surgical procedure in abdominal tract which is one of the reasons for the frequency of Gram-negative bacilli in our study because they are predominant gastrointestinal microflora.

Although diverse anaerobic populations are spread throughout the gastrointestinal tract, a relatively limited number of organisms are responsible for clinical infection in the surgical patient. Any event that may reduce the oxidation-reduction potential within the tissues encourages rapid anaerobic growth. Anaerobic infections in the surgical patient are typically associated with procedures that involve the gastrointestinal tract, but any anatomic site can also harbor anaerobic growth. Unlike nosocomial infections, which involve Gram-positive and -negative aerobic/facultative bacteria, anaerobic infections arise from the host's own endogenous flora, provided that appropriate host and environmental factors are present (11). In this study, most common anaerobic isolate was *B. fragilis* (13.4%) that is similar to other studies (9, 14). *Bacteroides* species are significant clinical pathogens and are found in most anaerobic infections. The bacteria maintain a complex and generally beneficial relationship with the host when remain in the gut, but when they escape this environment, can cause significant pathology, including bacteremia, SSI and other infections in multiple body sites (22). Decreased antibiotic susceptibility in anaerobic bacteria, especially in *B. fragilis* group, outnumbering of these organisms in gastrointestinal tract and polymicrobial nature of SSIs are factors for the presence and isolation of anaerobic bacteria in these infections (14).

Results of study carried out by Wolcott et al. for de-

tection of bacterial diversity in surgical site infection through molecular survey indicate high prevalence of anaerobic Gram-negative bacilli such as *B. fragilis* in these infections (10). In other studies, specimen's culture and phenotypic technique were applied for the prevalence evaluation of anaerobic bacteria in surgical site infections (12, 14). Since these organisms need particular condition for specimen's collection, transport, and cultures media, in some studies, the prevalence of anaerobic bacteria may be underestimated in surgical site infections. Correct specimen collection and accurate technique in culture workup can influence the isolation of anaerobic bacteria (14). In this study, we observed specimens collection from deep inside of infected site, immediately sealing of syringe, and primary specimen incubation in thioglycolate broth medium for 10 days, followed by subculturing in selective media supplied with antibiotics, sheep blood, vitamin K and hemin that can increase the chance of anaerobic bacteria isolation. SSIs often are polymicrobial infections and anaerobic bacteria mixed with aerobe and facultative bacteria (12, 14).

Amongst our study group, there were 14% monomicrobial (aerobic isolates only) and 82% polymicrobial cultures from which 28% were mixed aerobic-anaerobic infections, 56% aerobic/aerobic infections and the infections resulting merely from anaerobic bacteria were not observed, which were not correlated with the results of some researches (19) but similar to other works (9). Most of *Enterobacteriaceae* that isolated in this study have low susceptibility to β -lactam antibiotics and other antibiotics such as gentamicin and tetracycline. Extensive use of inappropriate antibiotics in empirical therapy can cause emergence of resistant bacteria strains, especially in healthcare centers. All isolated *Enterobacteriaceae* in this study were imipenem susceptible, which is consistent with the results of Seni et al. study (23).

Like other researches (9, 24), we reported *P. aeruginosa* with high level of resistance to tested antibiotics. Similar to other works, all *S. aureus* strains isolated in this study were oxacillin resistant and susceptible to vancomycin and linezolid (24, 25). In the past, β -lactam antibacterial agents were often used to treat anaerobic infections. In recent years, however, anaerobes have shown a tendency for development of resistance to these agents. All our *B. fragilis* isolates were resistant to penicillin, which is similar to findings of other researches (26). The most common mechanism of resistance to β -lactam antibiotics is β -lactamase production (22). On the other hand, these β -lactam antibiotics even along with beta-lactamase enzyme inhibitors such as amoxicillin in addition to clavulanic acid, also has lost a high percentage of their effectiveness against *B. fragilis* (27). This kind of resistance against β -lactam antibiotics plus β -lactamase inhibitor shows development of another method of resistance. The activities of cephalosporins vary greatly among individual agents of this family. In recent years, development of resistance of the *B. fragilis* group to cephalosporins has

been spreading (28). In this study, 61.5% of *B. fragilis* isolates and 100% of *C. perfringens* isolates were susceptible to ceftioxin, respectively.

Although all isolated strains of *B. fragilis* in this research are penicillin-resistant but resistance to other tested antibiotics also were observed that is in accordance with the results of other researches (26). In contrast, 32%, 69.2%, 69.2%, and 92.3% of *B. fragilis* isolates were sensitive to clindamycin, chloramphenicol, metronidazole, and imipenem, respectively, which are in agreement to the results of other works (26, 27). Penicillins are reportedly effective against non- β -lactamase-producing anaerobes. Among bacteria of *Clostridium* species, *C. perfringens* is highly susceptible to β -lactam because this bacterium does not produce β -lactamase. *C. perfringens* isolated in this study was 100% sensitive to penicillin while resistance to this antibiotic has also been reported (29). Presence of MDR (multidrug resistant) strains (MDR was defined as an isolate with resistance to 3 or more antimicrobial classes) (23), polymicrobial nature of these infections, and the role of anaerobic bacteria in surgical site infections can cause failure in antibiotic therapy. Therefore, for appropriate antibiotic therapy in prophylaxis and treatment of these infections, identification of causative microorganisms, including aerobic and anaerobic bacteria and frequency of high level antibiotic resistant strains in surgical site infections should be considered. For achieving these goals, close correlation between surgeon and microbiology laboratory is vital.

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Authors' Contributions

Mohammad Taghi Akhi: supervisor in all stages of the project; Reza Ghotaslou and Tahereh Pirzadeh: supervisor in isolation of aerobic bacteria; Samad Beheshtirouy: sampling of specimens; Mohammad Asgharzadeh; supervisor in molecular work; Babak Asghari, contribution in antibiogram performance; Naser Alizadeh and Ali Toloue Ostadgavahi: preparation of culture media; Vida Sorayaei Somesaraei: contribution in identification of isolates; Mohammad Yousef Memar: corresponding author and performing all stages of project.

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References

- Petrica A, Brinzeu C, Brinzeu A, Petrica R, Ionac M. Accuracy of surgical wound infection definitions—the first step towards surveillance of surgical site infections. *TMJ*. 2009;**59**(3-4):362-5.
- Reichman DE, Greenberg JA. Reducing surgical site infections: a review. *Rev Obstet Gynecol*. 2009;**2**(4):212-21.
- Kirby JP, Mazuski JE. Prevention of surgical site infection. *Surg Clin North Am*. 2009;**89**(2):365-89.
- Smith RL, Bohl JK, McElearney ST, Friel CM, Barclay MM, Sawyer RG, et al. Wound infection after elective colorectal resection. *Ann Surg*. 2004;**239**(5):599-605.
- Fry DE. The economic costs of surgical site infection. *Surg Infect (Larchmt)*. 2002;**3** Suppl 1:S37-43.
- Leaper DJ, van Goor H, Reilly J, Petrosillo N, Geiss HK, Torres AJ, et al. Surgical site infection - a European perspective of incidence and economic burden. *Int Wound J*. 2004;**1**(4):247-73.
- Gastmeier P, Sohr D, Rath A, Forster DH, Wischniewski N, Lacour M, et al. Repeated prevalence investigations on nosocomial infections for continuous surveillance. *J Hosp Infect*. 2000;**45**(1):47-53.
- Reddy BR. Management of culture-negative surgical site infections. *J Med Allied Sci*. 2012;**2**(1)
- Saini S, Gupta N, Griwan MS, Aparna. Surgical infections: a microbiological study. *Braz J Infect Dis*. 2004;**8**(2):118-25.
- Wolcott RD, Gontcharova V, Sun Y, Zischkau A, Dowd SE. Bacterial diversity in surgical site infections: not just aerobic cocci any more. *J Wound Care*. 2009;**18**(8):317-23.
- Edmiston CJ, Krepel CJ, Seabrook GR, Jochimsen WG. Anaerobic infections in the surgical patient: microbial etiology and therapy. *Clin Infect Dis*. 2002;**35**(Suppl 1):S112-8.
- Munez E, Ramos A, Espejo TA, Vaque J, Sanchez-Paya J, Pastor V, et al. [Microbiology of surgical site infections in abdominal tract surgery patients]. *Cir Esp*. 2011;**89**(9):606-12.
- Akhi M, R. Hajiloo G, T. Bohlooli. Isolation of vancomycin resistant *S. aureus* from postoperative infections. *Med J Tabriz Univ Med Sci*. 2006;**28**(2):9-13.
- Brook I, Frazier EH. Aerobic and anaerobic microbiology of surgical-site infection following spinal fusion. *J Clin Microbiol*. 1999;**37**(3):841-3.
- Mahon CR, Lehman DC, Manuselis Jr G. *Textbook of diagnostic microbiology*. New York: Elsevier Health Sciences; 2014.
- Wikler MA. *Performance standards for antimicrobial susceptibility testing: Sixteenth informational supplement*. Clinical and Laboratory Standards Institute; 2006.
- Clinical and Laboratory Standards Institute. *Methods for antimicrobial susceptibility testing of anaerobic bacteria*. Wayne: 2004.
- Pham AD, Mouet A, Pornet C, Desgue J, Ivascau C, Thibon P, et al. Enterobacteriaceae surgical site infection after cardiac surgery: the hypothetical role of vancomycin. *Ann Thorac Surg*. 2013;**96**(2):596-601.
- Giacometti A, Cirioni O, Schimizzi AM, Del Prete MS, Barchiesi F, D'errico MM, et al. Epidemiology and microbiology of surgical wound infections. *J Clin Microbiol*. 2000;**38**(2):918-22.
- Mahesh CB, Shivakumar S, Suresh BS, Chidanand SP, Vishwanath Y. A prospective study of surgical site infections in a teaching hospital. *J Clin Diagn Res*. 2010;**4**:3114-9.
- Owens CD, Stoessel K. Surgical site infections: epidemiology, microbiology and prevention. *J Hospital Infect*. 2008;**70**:3-10.
- Aldridge KE, Ashcraft D, O'Brien M, Sanders CV. Bacteremia due to *Bacteroides fragilis* group: distribution of species, beta-lactamase production, and antimicrobial susceptibility patterns. *Antimicrob Agents Chemother*. 2003;**47**(1):148-53.
- Seni J, Najjuka C, Kateete DP, Makobore P, Joloba ML, Kajumbula H, et al. Antimicrobial resistance in hospitalized surgical patients: a silently emerging public health concern in Uganda. *BMC Res Notes*. 2013;**6**(1):298.
- Bibi S, Chana GA, Siddiqui TR, Ahmed W. Pattern of Bacterial in Postoperative Wound and Their Sensitivity Patterns. *J Surg Pak*. 2012;**17**(4):164-7.
- Pal N, Guhathakurta R, Al-Jumaily EFA, Al-Mudallal NHA, Muhimen NAA, Al-Shaibany AAW, et al. Surgical site infection in surgery ward at a tertiary care hospital: the infection rate and the bacteriological profile. *J Pharm*. 2012;**2**(5):1-5.

26. Nakano V, Nascimento e Silva A, Merino VR, Wexler HM, Avila-Campos MJ. Antimicrobial resistance and prevalence of resistance genes in intestinal Bacteroidales strains. *Clinics (Sao Paulo)*. 2011;**66**(4):543-7.
27. Hedberg M, Nord CE, Escmid Study Group on Antimicrobial Resistance in Anaerobic Bacteria . Antimicrobial susceptibility of Bacteroides fragilis group isolates in Europe. *Clin Microbiol Infect*. 2003;**9**(6):475-88.
28. Snyderman DR, Jacobus NV, McDermott LA, Ruthazer R, Goldstein EJ, Finegold SM, et al. National survey on the susceptibility of Bacteroides Fragilis Group: report and analysis of trends for 1997-2000. *Clin Infect Dis*. 2002;**35**(Suppl 1):S126-34.
29. Tansuphasiri U, Matra W, Sangsuk L. Antimicrobial resistance among Clostridium perfringens isolated from various sources in Thailand. *Southeast Asian J Trop Med Public Health*. 2005;**36**(4):954-61.