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Detection of bacterial pathogens in surgical site infections and their antibiotic sensitivity profile

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ABSTRACT

Surgical site infections considered as a major problem in health care centers, resulting in extended length of stay, substantial associated morbidity and mortality, and high excess hospital cost. Thirty wound swabs were collected from patients who had developed postoperative wound infections at Rafidia Hospital-Nablus, Palestine. Bacterial isolates were identified according to standard microbiological methods. Antibiotics susceptibility test was applied for all isolated bacterial species. ERIC-PCR was carried out to determine the identity between isolated clones. The results of this research showed that the prevalence of pathogens among surgical site infections was 56.7%, 30%, 6.7%, 3.3% and 3.3% for E. coli, S. aureus, Klebsiella sp., Enterobacter sp., and Acinatobacter sp., respectively. E. coli isolates showed high resistance against Nalidixic acid (88.2%), Trimethoprim/Sulfamethoxazole (76.5%), Tetracycline (70.6%), Norfloxacin (64.7%) and Ciprofloxacin (58.5%). S. aureus showed high resistance against Nalidixic acid (88.9%), Norfloxacin (77.8%), Amoxycillin/clavulanic acid (77.8%), Kanamycin (66.7%) and Ciprofloxacin (55.6%). Methicillin resistant S. aureus (MRSA) accounted for 33.3% of a total of S. aureus isolates. Resistant to 3 or more antibiotics were detected in 94.1% (16/17) and 77.8% (7/9) of E. coli and S. aureus isolates, respectively. ERIC-PCR typing E. coli and S. aureus isolates showed that each was consisted of 4 ERIC-PCR clusters at a 50% similarity level. Indistinguishable and closely related strains were detected for both microorganisms. Results of this study might be important in provoking awareness to postoperative wound infections and further studies are needed to identify other pathogens responsible for SSIs and the source of infections. Using effective antibiotic policy will restrict further spread of postoperative wound infections.

Keywords: Surgical site infections, antibiotic resistance, ERIC-PCR profile, *E. coli*, *S. aureus*, MRSA.

INTRODUCTION

Surgical site infections (SSIs) are defined as infections that occur during one month after a surgical operation or one year after implant surgery and affecting either the injury site or near surgical injuries.[1] Since the publication of the guidelines for the prevention of surgical site infection in 1999 by the Center for Disease Control and Prevention (CDC), there has been a declining trend in SSI. According to the criteria put forth by the CDC, SSIs are classified as superficial incisional, deep incisional and organ/space SSIs.[2] Due to heterogeneous nature of these surgical infections, studies of the epidemiology of SSIs are very difficult. The incidence differs widely between surgical procedures, hospitals, patients and between surgeons.[3] Despite the technical advances in infection control and surgical practices, these infections still continue to be a major problem, even in hospitals with advanced modern facilities. The incidence of SSIs accounts as high as 20% among surgical patients, depending on the surgical procedure, the surveillance criteria used, and the quality of data collection.[4] Regardless the improvements in infection control techniques, surgical practices and substantial demands on healthcare resources, SSIs still considered as one of the major cause of morbidity and mortality.[2] Surgical site infections raise costs due to many

reasons such as extends the duration of hospitalization, additional diagnostic tests needed, more therapeutic antibiotic treatment, and other rarely reasons such as additional surgery.[5]

In most SSIs, the responsible pathogens are part of the patient's own endogenous flora. The most commonly isolated organisms are *Staphylococcus aureus* (*S. aureus*), coagulase-negative staphylococci (CNS), *Enterococcus* sp. and *Escherichia coli* (*E. coli*); however, the pathogens isolated depend on the surgical procedure.[4] Due to emergence antibiotic-resistant pathogens such as methicillin-resistant *S. aureus* (MRSA), multidrug resistant pathogens and others, this led to increase in incidence of SSIs. Other type of SSI pathogens may arise from exogenous sources such as health care workers, operating theatre environment, instruments and used materials. Such pathogens are predominantly aerobic microorganisms, in particular Gram-positive organisms such as staphylococci and streptococci.[2] In a retrospective review,[6] it was shown that 67% of implant infections were due to *S. aureus* and 68% of these cases were MRSA, while the prevalence of Gram-negative bacteria was only 6% among infections. In recent study,[7] a total of 137 samples obtained from patients had SSIs, 132 (96.4%) yielded bacterial growth and 139 bacterial isolates were obtained. The commonest organism was *S. aureus* (50.4%) followed by *E. coli* (23.02%), *Pseudomonas aeruginosa* (*P. aeruginosa*) (7.9%) and *Citrobacter* sp. (7.9%). Antimicrobial profile of Gram-positive isolates revealed maximum sensitivity to vancomycin, teicoplanin and linezolid, whereas among Gram-negative isolates meropenem, piperacillin-tazobactam, and amikacin were found to be most sensitive. *Staphylococcus aureus* strains showed a high degree of resistance for ampicillin (85.7%). Methicillin resistance was seen in 15.7% of all the *S. aureus* isolates. Gram-negative isolates showed high rate of resistance mainly to commonly prescribed agents like gentamicin, cotrimoxazole and ciprofloxacin.[7] *Staphylococcus aureus*, is considered as a major human pathogen and a predominant cause of SSIs worldwide with a prevalence rate ranging from 4.6% to 54.4%.[8] Infection with *S. aureus* is most likely associated with endogenous source as it is a member of the skin and nasal flora and also exogenous source with contamination from environment, surgical instruments or from hands of health workers.[9, 10] The prevalence of MRSA strains among SSIs ranged from 10%- 58.2%.[11-13] In other study, the prevalence of *S. aureus*, *Proteus mirabilis*, *E. coli*, *P. aeruginosa* and *Proteus vulgaris* among SSIs was 55.0%, 35.0%, 5.0%, 3.0% and 2.0%, respectively.[14] In other recent study, organisms associated with postoperative SSIs were *S. aureus* 22.4% followed by *Klebsiella* sp. 20.4% and *Proteus* sp. 18.4%, *E. coli* 12.2%, *Enterobacter* sp. and CNS each 8.2%, *P. aeruginosa* 6.1% and *Citrobacter* sp. 4.1%.[15] A total of 42 bacterial pathogens were identified of which 83.3% were from surgical sites and 16.7% were from blood stream infections. *Staphylococcus aureus* was the common pathogen accounting 26.2% followed by *E. coli* and CNS sp. Each represented by 21.4%. Approximately 100% of Gram-positive and 95.5% of Gram-negative bacterial isolates showed resistance against two or more antimicrobial agents.[16]

In Palestine, no previous studies concerning SSIs, this current study aimed to determine the prevalence of bacterial pathogens among patients with postoperative wound infections and to evaluate the antibiotic susceptibility pattern of these pathogens from Rafidia Hospital, Nablus-Palestine. In addition, to determine the molecular epidemiology of these pathogens isolates using ERIC-PCR technique.

MATERIALS AND METHODS

Sample collection and identification

A total of 30 bacterial isolates were collected using sterile cotton swabs from patients clinically diagnosed having SSIs at Rafidia Hospital-Nablus during February-April 2016. These samples were processed and identified as per standard microbiological techniques in Microbiology laboratory at An-Najah National University-Nablus, Palestine. The isolates were cultured on MacConkey and/or Eosin Methylene Blue agars, Mannitol salt agar, Blood agar and Triple sugar iron, Gram stain and biochemical tests were used as IMViC Tests (Indole production, Methyl red test, Voges-Proskauer test and Citrate utilization), catalse test, coagulase test, oxidase test, arginine hydrolysis, urea hydrolysis, H_2S production and motility test.

Antibiotic resistance

Antimicrobial resistance was determined according to the Clinical and Laboratory Standard Institute (CLSI) using the disk diffusion method.[17] Antibiotic disks (Oxoid) used were Ceftriaxone (CRO) 30µg, Norfloxacin (NOR) 10µg, Gentamicin (CN) 10 µg, Nalidixic acid (NA) 30µg, Levofloxacin (LEV) 5µg, Ciprofloxacin (CIP) 5µg, Amikacin (AK) 30µg, Cefuroxime sodium CXM (30µg), Tetracycline (TE) 30µg, Meropenem (MEM) 10µg, Kanamycin (K) 30µg, Trimethoprim/Sulfamethoxazole (SXT) 25µg, Cefotaxime (CTX) 30 µg, Ceftazidime (CAZ) 30µg, Vancomycin (VA) 30 µg, Teicoplanin (TEC) 30 µg and Amoxycillin/clavulanic acid (AMC) 30 µg. The

plates were incubated at 37°C for 18-24 hrs. Zones of inhibition were measured in millimeters using a caliper. Strains were classified as resistant or susceptible according to the criteria recommended by CLSI guidelines [17]. Oxacilllin (1μg) antibiotic disks (Oxoid) were used to detect MRSA. *Staphylococcus aureus* isolates were considered resistant if inhibition zones were ≤13 mm after incubation on 2% NaCl Mueller Hinton agar at 35°C for 24 hours. [17]

DNA extraction and ERIC-PCR

Bacterial DNA was prepared for PCR according to method described previously.[18] Briefly, cells were scraped off an overnight nutrient agar plate with a sterile loop, washed twice with 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8]), pellet was resuspended in 0.5 ml of sterile distilled H_2O and boiled for 10-15 min. The cells then were incubated on ice for 10 min. The debris pelleted by centrifugation at 11,500 X *g* for 5 min. DNA concentration was determined using spectrophotometer and samples stored at -20ºC until use for further DNA analysis.

ERIC (Enterobacterial repetitive intergenic consensus) PCR was performed using Primer ERIC1: 5`-ATG TAA GCT CCT GGG GAT TCA C-3` and Primer ERIC2: 5`-AAG TAA GTG ACT GGG GTG AGC G-3`. Each PCR reaction mix was performed in a final volume of 25 μl containing 12.5 μl of PCR premix with $MgCl_2$ (ReadyMix™ Taq PCR Reaction Mix with $MgCl_2$, Sigma), 0.8 μM of each primer, 150-200 ng of DNA template. In addition, the master mix was modified by increasing the concentration of dNTPs to 0.4 mM, 3mM $MgCl_2$ and 1.5U of Taq DNA polymerase. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 3 min at 94ºC was followed by 30 cycles of denaturation at 94°C for 50 s, annealing at 40°C for 50 s and extension at 72°C for 1 min, with a final extension step at 72°C for 5 min. The PCR products were analyzed by electrophoresis on 1.5 % agarose gel stained with Ethidium bromide. The gel image was scored using binary scoring system that recorded the presence and absence of bands as 1 and 0, respectively. A binary matrix was analyzed by the unweighted pair group method for arithmetic averages (UPGMA), using SPSS statistics software version 20 (IBM). The number of different bands in each fingerprint was considered for comparison of bacterial species as previously described,[19] based on the following criteria: "Indistinguishable" (No different band), "Closely related" (with 1 different band) "Possibility different" (with two different bands), "Different" (three or more different bands).

RESULTS

A total of 30 swab specimens were collected from patients with postoperative SSIs. In this study, the mean age of patients was 37.4 (1-80), and 63.3% (19/30) were males. Bacterial pathogens identified from these surgical site infections, age and sex of patients are presented in Table 1. Results of this research showed that the prevalence of pathogens among surgical site infections was 56.7%, 30%, 6.7%, 3.3% and 3.3% for *E. coli*, *S. aureus*, *Klebsiella* sp., *Enterobacter* sp., and *Acinatobacter* sp., respectively.

E. coli isolates showed high resistance against Nalidixic acid (88.2%), Trimethoprim/Sulfamethoxazole (76.5%), Tetracycline (70.6%), Norfloxacin (64.7%), Ciprofloxacin (58.5%). *S. aureus* showed high resistance against Nalidixic acid (88.9%), Norfloxacin (77.8%), Amoxycillin/clavulanic acid (77.8%), Kanamycin (66.7%) and Ciprofloxacin (55.6%). Methicillin resistant *S. aureus* were 33.3% of a total of *S. aureus* isolates. Results of antibiotics resistance against bacterial pathogens isolated from patients who had postoperative surgical site infections are presented in Table 2. Resistant to 3 or more antibiotics were detected in 94.1% (16/17) and 77.8% (7/9) of *E. coli* and *S. aureus* isolates, respectively.

ERIC-PCR typing of 17 *E. coli* isolates and 9 *S. aureus* isolates, which are believed to harbor different genes based on their antibiotic profiles, were genetically diverse and consisted of a heterogeneous population with a total of 4 ERIC-PCR profiles (clusters) at a 50% similarity level for both *E. coli* and *S. aureus* isolates. Results of ERIC-PCR profiles are presented in Figures 1, 2, 3 and 4. These results also showed that *E. coli* isolates numbered 16 and 17 were indistinguishable, while isolates 4 and15, 6 and 7, and 13 and 14 were closely related. For *S. aureus* isolates 2 and 9 were indistinguishable, while 1 and 5, and 6 and 8 were closely related.

Table 1: Surgical procedures and corresponding bacterial isolates from SSis patients

Pathogen	Operation (surgical site infection)	sex	Age	No. of Isolates
E. coli	Laparoscopic cholecystectomy	M	62	2
E. coli	Incisional hernia repair	F	57	1
E. coli	Debridement and skin graft	M	4	1
E. coli	Debridement of heel ulcer	F	65	1
E. coli	Perianal fistula operation	M	33	1
E. coli	A bone knee amputation	F	58	1
E. coli	Abdominal laparotomy due to intestines obstruction due to sigmoid CA	M	63	1
E. coli	Appendectomy	M	7 and 80	2
E. coli	Rightleg skin graft	M	48	1
E. coli	Umbilical and incisional hernia repair	F	57	1
E. coli	Perianal surgery	M	37 and 39	2
E. coli	Perianal fistulectomy	M	21	1
E. coli	Perianal abscess	M	1	1
E. coli	Right gluteal abscess	M	2	1
S. aureus	Laparotomy due to perforated sigmoid tumor	F	51	1
S. aureus	Hand surgery	F	62	1
S. aureus	Vertebral fixation	M	10	1
S. aureus	Skin graft	F	13	1
S. aureus	Rightinguinal hernia repair	F	39	1
S. aureus	Back lipoma excision	M	48	1
S. aureus	Groin abscess incision and drainage	F	1	1
S. aureus	Right-forearm graft	M	30	1
S. aureus	Right- thigh operation	F	1	1
Klebsiella sp.	Sigmoidectomy due to sigmoid carcinoma	M	61	1
Klebsiella sp.	Below knee amputation	F	22	1
Enterobacter sp.	Foot skin graft	M	18	1
Acinetobacter sp.	Craniotomy - chronic subdural haematoma	M	71	1

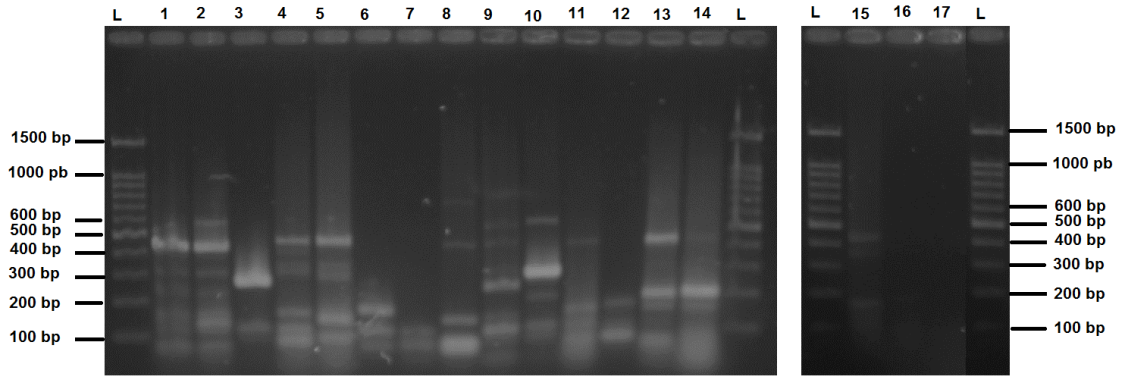

Figure 1: DNA fingerprints generated by ERIC-PCR analysis of 17 clinical *E. coli* isolates recovered from surgical site infections. Lanes L represent the ladder

Table 2: Antibiotic resistance profile for recovered bacterial pathogens

Microorganism	Antibiotic resistance n (%)*																	
	SXT	NOR	CIP	AK	K	TE	NA	CN	MEM	CTX	CRO	CAZ	LEV	CXM	OXA	VA	TEC	AMC
E.coli	13 (76.5)	11 (64.7)	10 (58.5)	4 (23.5)	7 (41.2)	12 (70.6)	15 (88.2)	6 (35.3)	0 (0.0)	6 (35.3)	6 (35.3)	6 (35.3)	N	N	N	N	N	N
Klebsiella sp.	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	2 (100)	1 (50)	2 (200)	2 (100)	2 (100)	1 (50)	1 (50)	N	N	N	N
Enterobacter sp.	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	N	N	N	N	N	N
Acinatobacter sp.	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	N	N	N	N	N	N
S. aureus	2 (22.2)	7 (77.8)	5 (55.6)	2 (22.2)	6 (66.7)	0 (0.0)	8 (88.9)	N	N	N	N	N	N	N	3 (33.3)	0 (0.0)	0 (0.0)	7 (77.8)

**Trimethoprim/Sulfamethoxazole, SXT; Norfloxacin, NOR; Ciprofloxacin, CIP; Amikacin. AK; Kanamycin, K; Tetracycline, TE; Nalidixic acid, NA; Gentamicin, CN; Meropenem, MEM; Cefotaxime, CTX; Ceftriaxone: CRO; Ceftazidime, CAZ; Levofloxacin, LEV; Cefuroxime sodium, CXM; Oxacilllin, OXA; Vancomycin, VA; Teicoplanin, TEC; Amoxycillin/clavulanic acid, AMC.*
N: Not detected.

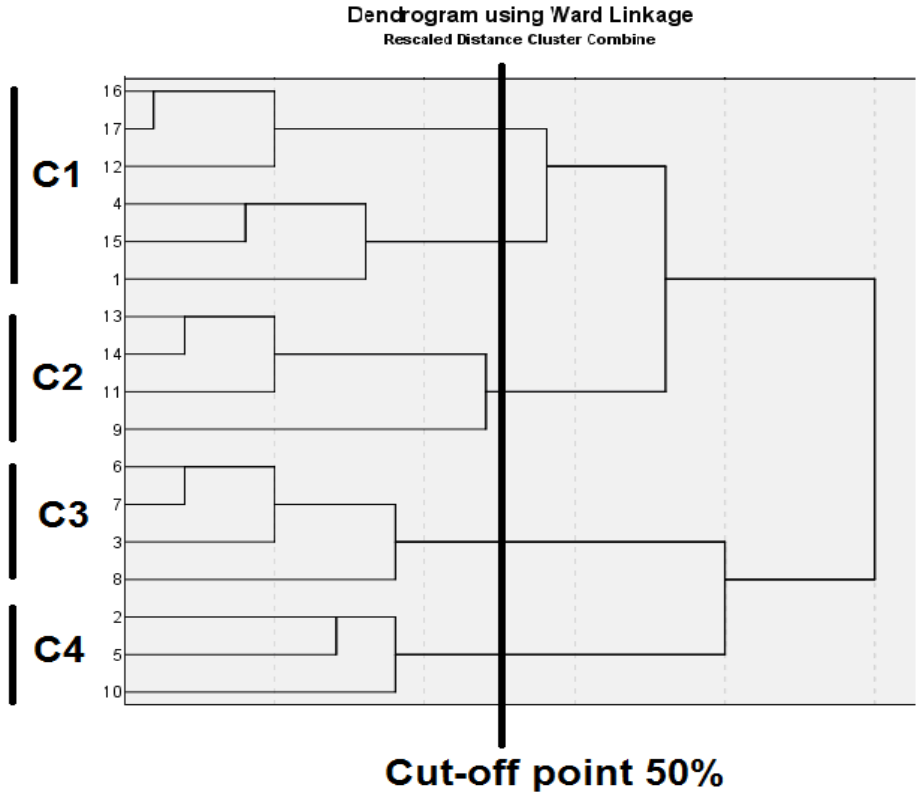

Figure 2: Dendrogram of 17 clinical *E. coli* isolates recovered from surgical site infections based on the UPGMA method using Ward's Method/ Squared Euclidean Distance by SPSS software version 20, derived from analysis of the ERIC-PCR-profiles at a 50% similarity level. C: Cluster

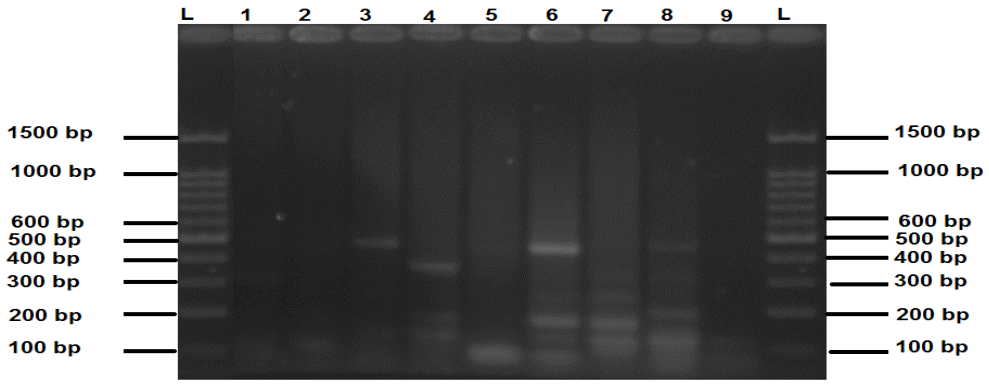

Figure 3: DNA fingerprints generated by ERIC-PCR analysis of 9 clinical *S. aureus* isolates recovered from surgical site infections. Lanes L represent the ladder

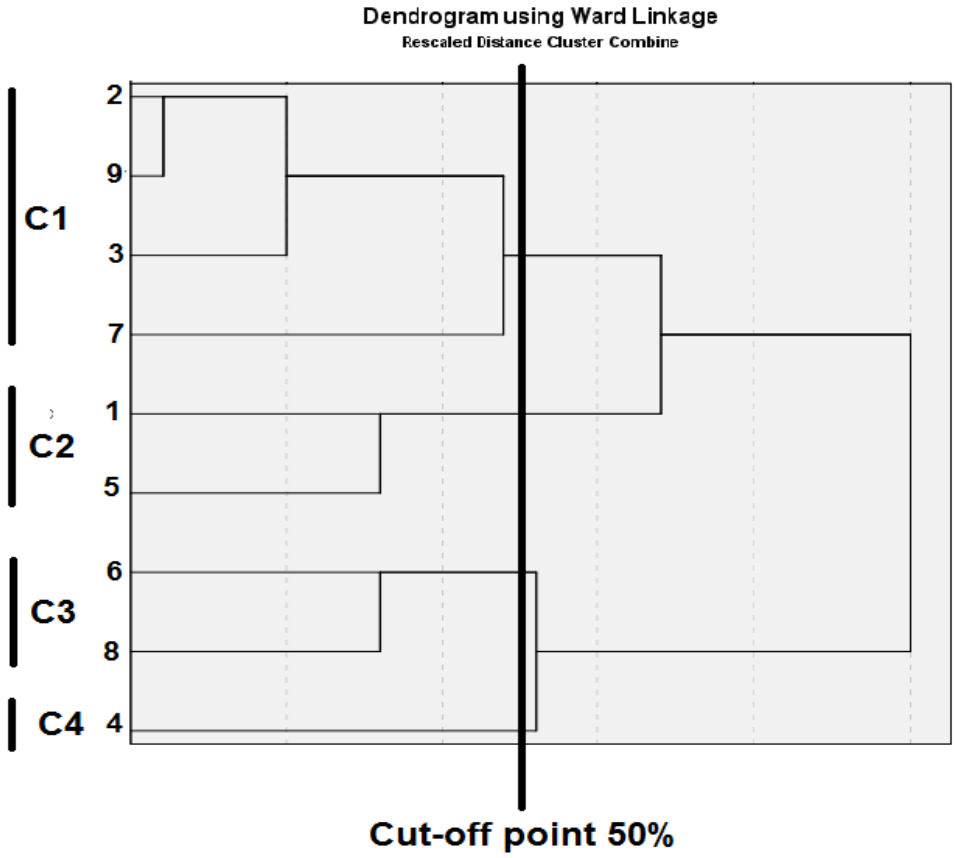

Figure 4: Dendrogram of 9 clinical *S. aureus* isolates recovered from surgical site infections based on the UPGMA method using Ward's Method/ Squared Euclidean Distance by SPSS software version 20, derived from analysis of the ERIC-PCR-profiles at a 50% similarity level. C: Cluster

DISCUSSION

Postoperative hospital infections are considered a major problem in health care centers, resulting in extended length of stay, substantial associated morbidity and mortality, and high excess hospital cost.[14] These infections have been reported to be one of the major common causes of nosocomial infections and are accounting for 20% to 25% of all nosocomial infections worldwide.[20] It is estimated that SSIs develop in 2%-5% of the 16 million patients undergoing surgical procedures each year in the United States.[21] In spite of technological advances in surgery and wound management, wound infections still regarded as the one of the most common hospital infection mainly in patients undergoing surgery.[22]

In the current study, *E. coli* isolates was the most common pathogen (63.3%) in postoperative wound infections. In other studies the incidence *E. coli* in postoperative wound infections ranged from 5%-23%.[7,14,15] It was also reported to be the commonest Gram-negative bacteria isolated in several other studies.[7,8,14,16] Infection with *E. coli* is most likely associated with endogenous source as it is a member of intestinal normal flora and this might explain the finding of this pathogen in most of operations related to the digestive system. Infection with *S. aureus* is most likely associated with endogenous source as it is a member of the skin and nasal flora and also exogenous source with contamination from environment, surgical instruments or from hands of health workers.[9,10,23] In previous study, it was shown that 4 out of 6 operating rooms in this hospital were contaminated with *S. aureus*, and the average number of *S. aureus* in these rooms ranged from 668-4438 CFU/m2 using passive air sampling.[24]

Staphylococcus aureus is a major cause of infection in both healthcare and community settings. This pathogen was the only Gram-positive bacteria isolated from the postoperative wound infections in this study. Findings of the current study in this respect were in contrast to previously reported results, where *S. aureus* was a major cause of

SSIs.[6,7,14] In these studies prevalence of *S. aureus* ranged from 50%-67%, however, the findings of Gelaw et al. (2014) on the prevalence of this pathogen among SSIs was 22% and consistent with that found in the current study.[15] In the current study, around 30% (3/9) of *S. aureus* were MRSA and these constitute 10% (3/30) of the total SSIs isolated pathogens. In previous studies, the prevalence of MRSA strains among SSIs ranged from 10%-58.2%.[11-13]

In this study, most isolates of *E. coli* and *S. aureus* showed multi-drug resistant to the commonly prescribed antibiotics such as Nalidixic acid, Trimethoprim/Sulfamethoxazole, Tetracycline, Norfloxacin, Ciprofloxacin and Kanamycin. This is probably due to selective pressure resulting from uncontrolled, extensive incorrect and misuse of these antibacterial agents in hospitals as well as in the country as a whole. This is promoted by the lack of national antibiotic policy and over-the-counter antibiotic availability in Palestine.[25] All *E. coli* isolates that were susceptible to Meropenem and less resistant to Ceftazidime and Amikacin while *S. aureus* being sensitive to vancomycin, Teicoplanin and Tetracycline; this could be explained by the fact that some of these antibiotics are rarely used in the hospitals due to their high cost, thus, they are rarely misused.

ERIC-PCR typing of *E. coli* and *S. aureus* showed that each of these pathogen isolates where divided into 4 clusters. Analysis of ERIC-PCR profiles showed indistinguishable similar patterns in 2 isolates of each of *E. coli* and *S. aureus* isolates. Some of the other isolates of both pathogens were closely as revealed by ERIC-PCR profiles. Variation in ERIC-PCR profiles for closely related isolates could be due to genomic DNA alterations such as mutations and activation of motile elements as transposons.[19] Other useful methods can be used to study molecular epidemiology of these pathogens such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST).

CONCLUSION

To our knowledge, up to now, this is the first study documented the types of pathogens responsible for postoperative wound infections in Palestine. Effective antibiotic policy in various health settings will restrict the further spread postoperative wound infections. The aforementioned data might be important in provoking awareness to postoperative wound infections and further studies are needed to cover a wide range of surgical procedures in different health care settings and source of infections. Detection of DNA fingerprint patterns by ERIC-PCR is simple and suitable method for epidemiological studies.

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