INTRAVENOUS LINE COLONIZATION IN PATIENTS RECEIVING CYTOTOXIC DRUGS

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ABSTRACT

Background

Hospital acquired infections are infections that develop during the hospital stay; they mainly include urinary tract, respiratory tract or blood stream infections.

Objectives

To investigate microbial colonization of intravenous cannula of cancers patients receiving cytotoxic drugs.

Materials and Methods

Colonization of intravenous cannulas was investigated by cultivation of the device surface and lumen on different culture media under aerobic incubation. The isolates were identified based on cultural, morphological and biochemical activities. The susceptibility of the isolates to antimicrobial drugs was investigated using Bauer-Kirby disk diffusion method and the bacterial isolates were further investigated for their ability to form biofilm.

Results

From 200 cultivated intravenous cannulas, microbial colonization was detected among 26% and 11% of intravenous cannulas from cancer and non-cancer patients respectively; Out of 37 isolates, 34 (91.9 %) were Gram-positive bacteria, 2 (5.4 %) Gram-negative bacteria and one (2.7 %) was a Candida species. The main bacterial isolates were bacteria belonging to different coagulase negative staphylococci species such as *Staphylococcus epidermidis*, *S. hominis* and others. Few faecal organisms were isolated including a single isolate of each *Klebsiella pneumoniae* and *Escherichia coli*. All bacterial isolates were able to form biofilm and most showed to have biofilm-related *icaA* and *icaD* genes.

Conclusion

We detected microbial colonization of intravenous cannulas in 18.5% of all cultivated devices. We observed more colonization in cancer patients (26%) in comparison to the control group (11%). Most of the isolates were species of CoNS that were able to form biofilm.

Keywords: *Intravenous line colonization, Hospital infection, Nosocomial, CoNS, Biofilm.*

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INTRODUCTION

Nosocomial infections or hospital-acquired infections are infections acquired in hospitals or in healthcare services unit that appear 48 hours or more after hospital admission; these also include infections acquired in the hospital but appearing after discharge or occupational infection among staff [1]. Nosocomial infections occur worldwide; an approximate of three million infections are identified in the European Union each year [2]. Nearly 60% to 70% of nosocomial infections are associated with the use of different medical devices [3], while the contaminated hands of medical personnel are considered as the main way for nosocomial infection spread [4].

Medical device related infection (MDRI) is an infection in a patient with a medical device (intravascular catheter, endotracheal tube or indwelling urinary catheter) that was in use for at least 48 hours before the onset of infection. The wide use of indwelling devices in hospitalized patient has increased the incidence of MDRI, especially blood stream infections which originate from microbial colonization of the intravascular catheters [5]. Intravascular catheters are essential in modern day medical practice, especially in intensive care units but they put the patients at risk local infection and catheter-related bloodstream infections (CRBSI). Risk factors for the development of catheter-related infections included immune compromised status, duration of the catheter *in situ*, femoral venous cannulation, and the triple lumen catheters [6].

Microorganisms commonly associated with peripheral vascular and central venous catheter infection are members of coagulase-negative staphylococci (CoNS), *Staphylococcus aureus*, different species of aerobic gram-negative bacilli, and *Candida albicans* [7]. Microorganisms that colonize the catheters surface may originate either from the skin, migrating along the external surface of the device, or from the catheter hub during manipulation and migrating along the inner lumen of the catheter [8]. After colonization, the organisms enter into the blood stream and cause CRBSIs. It is estimated that 200,000 CRBSIs occur annually in the United States and nearly 87% of these were caused by staphylococci [9, 10]. When the organisms colonize the tip of the catheters this cause local infection and this is called catheter-related local infections (CRLI) [11].

Bacteria like *S. epidermidis* –a member of normal skin flora– has affinity to adherence to plastic or smooth surfaces and form biofilm is the most frequently isolated species from medical devices [12]. Biofilms consist of multiple layers of bacterial cells embedded into a glycocalyx that protect the bacteria against the immune system and effect of antibiotic agents [13].

Biofilm formation is detected by qualitative methods, such as the tube adherence test [14] or Congo red agar method [15], and quantitative methods such as the micro-well plate assay [16]. The ability to form biofilm lies in the presences of certain genes among *ica* genes. The *icaA* and *icaD* have been reported to play a significant role in biofilm formation. The *icaA* gene encodes N-acetylglucosaminyltransferase, an enzyme involved in intracellular adhesion protein synthesis. Additionally, *icaD* has been reported to a play a critical role in the maximal expression of this enzyme [17-19].

This study was carried out to identify microbial colonization of intravenous cannula in cancer patients receiving cytotoxic therapy and to test the antimicrobial susceptibility and biofilm formation of the isolates.

MATERIAL AND METHODS

Two hundred intravenous cannulas (IVC) were collected from 200 inpatients from the period of March to November 2014. One hundred samples were collected from patients suffering from different cancers admitted to receive parenteral cytotoxic therapy in Hiwa Teaching Hospital and the rest were collected from non-cancer, children and adult patients admitted to the Pediatric and General Medical Teaching Hospital respectively for diseases other than first group. The study was approved by "The Ethics Committee of the Faculty of Medical Science, School of Medicine". Informed consent about the study was obtained from each patient agreed to participate in the study before the cannulas were removed. The IVC were extracted and collected into sterile screw cap containers using sterile gloves after disinfecting the skin around the cannula site with 10% povidone iodine.

Cultivation, Isolation and Antimicrobial Susceptibility Test

Cultivation of IVC tips were performed by semi-quantitative and quantitative methods; for semi-quantitative method, the IVC tip was streaked entirely on blood agar (HiMedia®, India), MacConkey agar (HiMedia®, India), mannitol salt agar (Oxoid, USA) and sabouraud dextrose agar (Salucea VOF Dutch

Technology, the Netherlands) and the inoculated media were incubated at 37°C for 18-24 h [20]. For quantitative method, we flushed the internal lumen of the cannula with two mL of brain heart infusion broth (Lab M, UK) into a tube and vortexed it together with 2 cm cut of the cannula tip for 15 s, the broth was then incubated at 37° C for 18-24 h. We spread 0.1 mL of cultured broth on the previously mentioned media [21]. We identified the isolates on the basis of colony characteristics, Gram staining properties, biochemical activities [22, 23] while we used API® 32 Staph system (BioMérieux) and Vitek® 2 (BioMérieux) system for final identification of the bacterial of the isolates.

We performed antimicrobial susceptibility test according to Bauer-Kirby disk diffusion method [24] using antimicrobial disks (Bioanalyse®, Turkey) and the results were evaluated according to the Clinical and Laboratory Standard Institutes guidelines [25].

Biofilm formation

We tested the ability of the bacterial isolated to form biofilm by three methods; Congo red agar method (15), tube method using tryptic soy broth (Salucea VOF Dutch Technology, the Netherland) in glass tube with crystal violet as a staining dye [14], and micro-well plate assay where 96-well plate was used with crystal violet as a staining dye and the results were red at OD of 570 nm [16, 26, 27].

Detection of biofilm genes icaA and icaD

Overnight culture of isolates in tryptic soy broth (Salucea VOF Dutch Technology, the Netherland) was used for genomic DNA extraction using Geneaid™ DNA isolation kit. PCR amplification of *icaA* and *icaD* genes [19] was performed using two primers sets (macrogen, Korea); icaA-For (5′-TCTCTTGCAGGAGCAATCAA-′3), icaA-Rev (5′-TCAGGCACTAACATCCAGCA-′3), icaD-For (5′-ATGGTCAAGCCCAGACAGAG-′3) and icaD-Rev (5′-CGTGTTTTCAACATTTAATGCAA-′3). PCR was carried using reaction mixture Prime Taq™ Premix (Kiagen-biotech, IRAN), primers and nuclease free distilled water. Amplification conditions were according to Aricola et al. (2001) using Techne™ thermal cycler TC-512. We resolved the PCR products using 1% agarose gel electrophoresis (biobasic, Canada) and the ethidium bromide stained DNA band were documented with an UV trans-illuminator (LabTech, Korea).

RESULTS

One hundred intravenous cannulas were from cancer patients and this consisted of 56 males and 44 females while the control group consisted of 32 males and 68 females. The age of the cancer patients ranged from 2 to 88 years (average 36.88 y) while for the control group the age ranged between 1 to 81 years (average 25.58 y).

Colonization of Intravenous Cannula

We detected microbial growth from 26 (26%) IVC in the cancer group and from 11 (11%) IVC in the control group, which was statistically significant ($p < 0.05$). Table 1 shows the results of the growth in relation to different characteristics of the study groups. Irrespective to the study groups, the relation of gender to the growth was not statistically significant while this relation was significant only in the control group ($p= 0.002$).

The duration of hospitalization, the age of IVC and the average daily manipulations of the IVC was reported for each patient. The relation of these to growth was not significant except the IVC age (irrespective to the study group) with a $p < 0.001$. Most of IVC (80%) were inserted on the dorsum of hand, the arm (12%) and the foot (8%). The relation of growth to the site of the inserted IVC was statistically non-significant irrespective to and according to the study groups.

Local IVC problems like erythema, pain and swelling were reported in 132 (66%) of the patients in the study. The relation of growth to IVC problem was statistically non-significant irrespective to and according to the study groups.

Other data such as absolute neutrophil count, presence of fever during the sampling and current antimicrobial treatment during sampling were acquired; the relation of growth to these data were all none statistically significant irrespective to the study groups or in both groups.

Table 1. The growth results from the intravenous cannulas in relation to different characteristic of the patients and the cannula.

Characteristic	Cancer group		Control group		Total	p value
	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)		
Gender						
Female	8	36	9	59	112 (56)	0.002 *
Male	18	38	2	30	88 (44)	
Mean day stay in hospital	5.61	6.12	4.36	3.11		
Mean i.v. cannula age (day)	2.65	2.18	3.9	1.93		0.001**
Mean daily i.v. cannula manipulation	15.46	13.87	15.81	12.11		
Cannula position						
Hand	17	48	11	84	160 (80)	
Arm	6	13	0	5	24 (12)	
Foot	3	13	0	0	16 (8)	
Cannula problem						
Yes	26	66	5	35	132 (66)	
No	0	8	6	54	68 (34)	
Neutrophil count						
Increased	11	20	0	4	35 (17.5)	
Decreased	5	21	1	6	33 (16.5)	
Normal	10	29	1	11	51 (25.5)	
Not available	0	4	9	68	81 (40)	
Fever						
Yes	6	18	1	10	35 (17.5)	
No	20	56	10	79	165 (82.5)	
Received antibiotics						
Yes	18	57	5	50	130 (65)	
No	8	17	6	39	70 (35)	

* within control group , ** Irrespective to study group

Growth Results

From the 200 IVC cultivated under aerobic conditions, 37 yielded a positive growth (26 from cancer group and 11 from the control group). Only one IVC yielded growth with semi quantitative cultivation technique and the remaining growth were isolated with quantitative method. Table 2 shows the isolated microorganisms; CoNS was the most predominant with *S. epidermidis* and *S. hominis* leading the isolates. Enteric bacteria such as *E. coli*, *K. pneumoniae* from the control group and *Enterococcus faecium* from the cancer group were also isolated. Three Kocuria spp. and a single Candida spp. was also isolated.

Antimicrobial Susceptibility response of the Isolates

Antimicrobial susceptibility testing of the isolates showed mainly resistance response. All *S. epidermidis* were resistant to methicillin, cefoxitin and amoxicillin-clavulanate; while most were susceptible to vancomycin, imipenem, levofloxacin and cotrimoxazole. *Staphylococcus hominis* were resistant to cefoxitin (67%) and 83.3% resistance to methicillin, azithromycin, cefotaxime, levofloxacin, and amoxicillin clavulanate, while all were susceptible to amikacin, cefipime. Furthermore; all *S. hemolyticus* were showed resistant to methicillin and cefoxitin and 75% were resistant towards ciprofloxacin, levofloxacin and cefipime. All *S. hemolyticus* were susceptible to imipenem while susceptibility to azithromycin, amikacin and amoxicillin-clavulanate reached 75 %.

The overall activities of antimicrobial agents against gram-positive isolates are shown in Table 3. The effective agents were amikacin, imipenem, levofloxacin, ciprofloxacin and vancomycin, while more resistance was detected to methicillin, cefoxitin. Supplementary data contain details of antimicrobial response of the isolates.

Biofilm Formation

Biofilm formation was tested for all 36 bacterial isolates. All the isolates were shown to be able to form biofilm detected at least with a method, Table 2. Comparing the three detection methods, we found that micro-well plate assay showed higher positive results (32 or 88.9 % of isolates) in comparison with the other two methods (28 or 77.7% for tube method and 27 or 75% for Congo red). The degree of biofilm formation was also compared in the three methods; we found that most of the isolates showed weak biofilm formation with Congo red agar method (74%) while the other methods detected more moderate to strong biofilm response, Table 5.

Molecular Detection of Biofilm Genes

We used PCR to detect biofilm gene *icaA* and *icaD* among the 34 gram-positive isolates (Figure 1, 2). Table 5 shows the results of *icaA*, *icaD* or both genes; we detect *icaD* gene in 94.1% of the isolates in comparison to 82.4% for *icaA*. To confirm PCR products, DNA sequencing was performed with three PCR products for each *icaA* and *icaDi* PCR products. DNA sequence queried with BLAST service available at National Center for Biotechnology (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and revealed the correct products (supplementary data not shown).

Table 2. The isolated microorganisms from cultivation of intravenous cannulas (n=200) under aerobic condition from both study groups and their ability to form biofilm.

Bacterial species	No. (%) of isolates	Congo red agar No. (%)	Tube method No. (%)	Micro-well plate assay No. (%)
Staphylococcus epidermidis	8 (21.6)	7 (87.5)	8 (100)	8 (100)
Staphylococcus hominis	6 (16.2)	6 (100)	5 (83.3)	5 (83.3)
Staphylococcus hemolyticus	4 (10.8)	4 (100)	3 (75)	4 (100)
Staphylococcus lugdunensis	3 (8.1)	2 (66.6)	3 (100)	3 (100)
Kocuria variant/rosea	3 (8.1)	0 (0.0)	1 (33.3)	3 (100)
Staphylococcus xylosus	2 (5.4)	1 (50)	2 (100)	2 (100)
Micrococcus luteus	2 (5.4)	1 (50)	1 (50)	0 (0.0)
Enterococcus faecium	2 (5.4)	2 (100)	0 (0.0)	1 (50)
Staphylococcus chromogenes	1 (2.7)	0 (0.0)	1 (100)	1 (100)
Staphylococcus warneri	1 (2.7)	1 (100)	1 (100)	1 (100)
Staphylococcus auricularis	1 (2.7)	0 (0.0)	1 (100)	1 (100)
Staphylococcus lentus	1 (2.7)	1 (100)	0 (0.0)	1 (100)
Escherichia coli	1 (2.7)	1 (100)	1 (100)	1 (100)
Klebsiella pneumoniae	1 (2.7)	1 (100)	1(100)	1 (100)
Candida spp.	1 (2.7)			
Total	37 (100)	27 (75)	28 (77.7)	32 (88.9)

Table 3. Overall Antimicrobial response among all the Gram-positive isolates (n=34).

Antimicrobial agent	Susceptible No. (%)	Intermediate No. (%)	Resistant No. (%)
Amikacin	26 (76.6)	1 (2.9)	7 (20.6)
Amoxicillin/Clavulanate	20 (58.82)	1 (2.96)	13 (38.24)
Azithromycin	16 (47.05)	0 (0.00)	18 (52.94)
Ciprofloxacin	22 (64.705)	1 (2.96)	10 (29.41)
Cotrimoxazole	19 (55.88)	1 (2.96)	14 (41.17)
Cefotaxime	13 (38.23)	2 (5.88)	19 (55.88)
Cefipime	23 (67.65)	1 (2.96)	10 (29.41)
Cefoxitin	6 (17.65)	0 (0.00)	28 (82.35)
Imipenem	26 (76.47)	0 (0.00)	8 (23.53)
Levofloxacin	24 (70.59)	2 (5.88)	8 (23.53)
Methicillin	5 (14.7.5)	0 (0.00)	29 (85.29)
Meropenem	19 (55.89)	2 (5.88)	13 (38.24)
Vancomycin	24 (70.59)	0 (0.00)	10 (29.41)

Table 4. Comparison of different phenotypic methods for biofilm detection.

Methods	Biofilm producer No. (%)		
	Weak	Moderate	Strong
Congo red agar method	20 (74)	4 (14.8)	3 (11.1)
Tube method	14 (50)	5 (17.9)	9 (32.1)
Micro-well plate assay	6 (18.7)	11 (34.37)	15 (46.9)

Table 5. PCR detection of biofilm formation genes *icaA* and *icaD* among the Gram-positive isolates.

Bacterial species	Number of isolates	Positive *icaA* gene No. (%)	Positive *icaD* gene No. (%)	Positive *icaA and icaD* genes No. (%)
Staphylococcus epidermidis	8	7 (87.5)	8 (100)	7 (87.5)
Staphylococcus hominis	6	4 (66.6)	6 (100)	4 (66.6)
Staphylococcus hemolyticus	4	3 (75)	4 (100)	3 (75)
Staphylococcus lugdunensis	3	3 (100)	3 (100)	3 (100)
Kocura variant/rosea	3	3 (100)	3 (100)	3 (100)
Staphylococcus xylosus	2	1 (50)	2 (100)	1 (50)
Micrococcus luteus	2	2 (100)	1 (50)	1 (50)
Enterococcus faecium	2	1 (50)	1 (50)	1 (50)
Staphylococcus chromogenes	1	1 (100)	1 (100)	1 (100)
Staphylococcus warneri	1	1 (100)	1 (100)	1 (100)
Staphylococcus auricularis	1	1 (100)	1 (100)	1 (100)
Staphylococcus lentus	1	1 (100)	1 (100)	1 (100)
Total	34	28 (82.4)	32 (94.1)	27 (79.4)

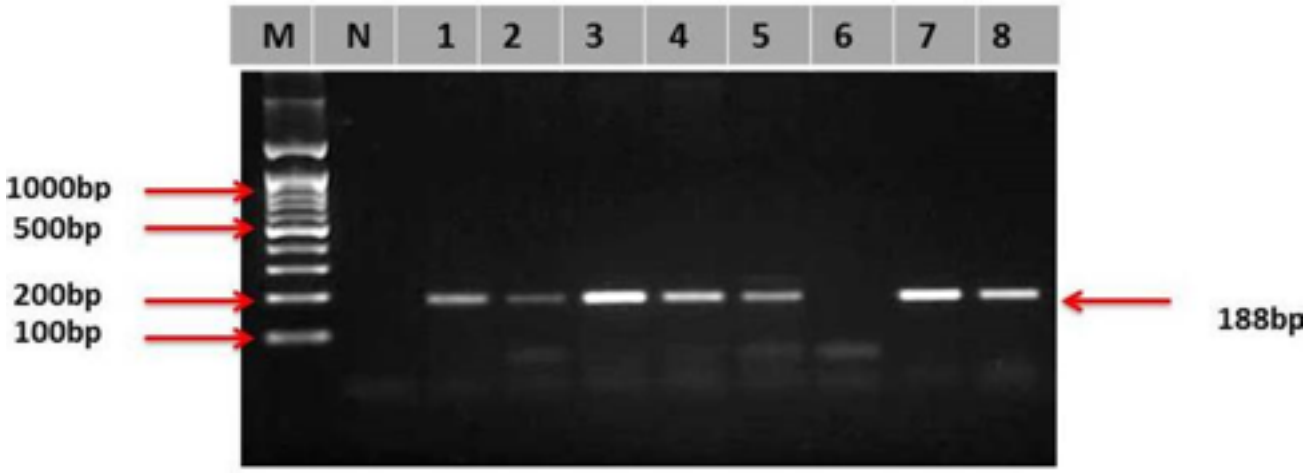

Figure. 1 Agarose gel electrophoresis (1.5%) of PCR amplification of *icaA* gene. M: DNA markers, N: negative control, lane 1 & 2: *S .epidermidis* products, lane 3 & 4: *S. hominis* products, lane 5 & 6: *S. hemolyticus* product, lane 7: *S. xylosus* product and lane 8: *S. lugdunensis* product.

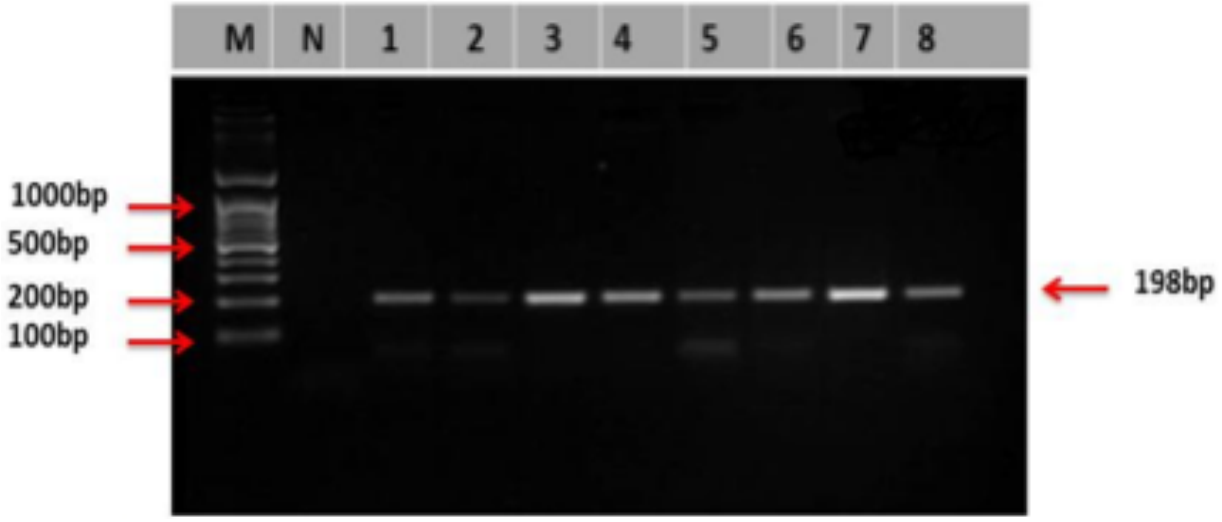

Figure 2. Agarose gel electrophoresis (1.5%) of PCR amplification of *icaD* gene. M: DNA markers, N: negative control, lane 1 & 2: *S .epidermidis* products, lane 3 & 4: *S. hominis* products, lane 5 & 6: *S. hemolyticus* product, lane 7: *S. xylosus* product and lane 8: *S. lugdunensis* product

DISCUSSION

Here, we investigated the possibility of colonization of intravenous cannulas (IVC) by bacteria or fungi in cancer patients receiving cytotoxic treatment. The IVC were removed from the patients when no more needed, or following a local problem. We detected growth from 26% of IVC in the cancer patients and in 11% from the control group. This difference may be explained to be due the effect of their cancer diseases or cytotoxic therapy affecting their immune system. Immune deficiency is an important predisposing risk factor for the development of catheter-related infections [6].

Ghotaslou et al., [28] investigated the relation of intravascular colonization with BSI, and reported only 13 colonized catheters (8.7%) out of 150 arterial or venous catheters in cardiac surgery in ICU. Catheter colonization of 7.5% and 9.3% was also reported in two other studies [29, 30]. These differences may be related to the type of catheter, type of disease, or related to the techniques during device insertion or manipulation in the hospitals. In another study on surgical ICU of 93 patients, the rate of catheter related infection was 34% [31]. Furthermore, Tomanović and Mirović [32] showed that out of 289 catheter culture, 217 cases were positive and rate of catheter colonization was (75%). Mozaffari

et al. found 66.7% (44 out of 66 catheter) growth which seems to be too high compared with our results, and also recorded *S. epidermidis* as the most frequent isolate (34 %) [33] and in a study from the United States, 260 patients were assessed and colonization was detected from 21% catheters [34].

The rate of colonization of intravascular devices is different from various studies. The rate of IVC colonization in our study (18.5%) is lower or near many of the previous studies. Awareness of infection control, hand washing, skin preparation before catheter insertion, and removing unnecessary catheters all play a role in the colonization rate.

In our study, the relation of gender to the growth was statistically significant only in control group where growth observed from IVC of nine females and two males. Few studies showed an increase colonization rate among females (28, 35) although such results was not reported by others [36, 37].

The duration of hospitalization, the age of IVC and the average daily manipulation of the IVC were analyzed against growth; these relations were statistically not significant in both cancer and control group, but a statistically significant relation was observed only with the age of IVC irrespective with the study group. Ghotaslou et al. investigated this relationship and found that the longer the catheter remains, the more likely colonization and infection occurs [28]. Many studies concluded that devices are possible to be infected during manipulation or drug administration and some devices are infected from the infusate [6, 38].

All the IVC that yield a positive growth from the cancer patients [26] were from patients with local problem at the insertion site of IVC such as erythema, pain and swelling indicating the possibility of an infection. While colonization was detected in only five patients with local IVC problems from control group indicating the possibility of colonization even in the absence of local feature of inflammation [39].

Many patients were receiving antimicrobial treatment when the devices were removed for cultivation. Antimicrobial treatment was reported among 75% of the cancer patients and in 56% of the control group and many of those colonized IVC were from patients on antimicrobial treatment indicating that antimicrobial treatment might have no effects on the colonized bacteria due to their of biofilm state that give the bacteria a natural resistance toward antimicrobial or antiseptics [40, 41]. Many antimicrobial resistances were reported from local hospital inpatients indicting a local antibiotic resistance toward many agnets [42, 43].

What we observed is that, there is a high possibility of colonization of IVC by bacteria and if very strict rules during device insertion or manipulation are not followed, these devices become a source of infection such as local infection or bacteremia.

The quantitative method for cultivation of bacteria from the IVC was better than semi-quantitative method as the latter method yield only a single isolate from the external surface of the catheter rather than intraluminal colonization [20, 21, 44]. Prominent skin microbiota, *S. epidermidis* and other related CoNS species were the main colonizers, these organisms were also reported by others although with various ratios [32, 45, 46]. Finding such organisms are due to their presences as skin microbiota [47] and their ability to form biofilm [12, 48, 49].

Although it was not our aim to investigate if IVC colonization was associated with bacteremia, the possibility of this cannot be ruled out. CoNS are of low virulence but can make problems in immunocompromised patients such as the cancer patients [50].

The isolation of enteric organisms such as *Escherichia coli*, *Klebsiella pneumoniae* and *Enterococcus faecium* indicate contamination by these organisms during insertion or manipulation of IVC. Insertion and manipulations of IVC should be done with extra care and with precaution to prevent such problems [51, 52]. The isolation of three Kocuria spp. indicates a depressed immune system that favorers colonization unusual organisms [53, 54].

The isolates were resistant to many antimicrobial agents such as methicillin, cefoxitin and amoxicillin-clavulanate and multidrug resistance bacteria were observed in many isolates; many of these antimicrobials were frequently used in the studied hospitals. Many of the isolates were susceptible to vancomycin, imipenem, levofloxacin and cotrimoxazole. Resistance will make treating infection with such organism difficult; in addition, the ability to form biofilm increases their insusceptibility to the drugs [55-57]. The resistance response reflects the resistance among bacteria in the community or in the local hospitals [58, 59].

Colonization of IVC and development of infection has been related to the capacity of these organisms

to produce biofilm [60]. All isolates in this study were able to form detected biofilm at least with one of the three mentioned methods. The micro-well plate assay method was better than other two methods; the sensitivity of these techniques was similar to a previous study findings [61]. Biofilm *icaD* gene was detected more than *icaA* gene among the isolates (94.1% to 82.4%) indicating the presence of these genes and their expression among many of the isolates. These genes beside other genes, are needed and their expression is essential for biofilm formation [62]. The detection of such genes and biofilm detection by these organisms reveals that biofilm formation is an integral property of many bacteria and these bacteria utilize many surfaces to form biofilm [60, 63, 64].

In conclusion, we detected microbial colonization of IVC in 18.5% of all cultivated devices. We observed more colonization in cancer patients (26%) in comparison to the control group (11%). Most of the isolates were species of CoNS and were able to form biofilm.

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