COMPRISAL EFFECT OF PROBIOTIC AND ANTIBIOTICS ON BIOFILM FORMING COAGULASE NEGATIVE *STAPHYLOCOCCI* CLINICAL ISOLATES

Marwa A. Al-Asady[1*], Jasim H. Makhrmash[1] and Ahmed M. Taqi[2]

[1]Department of Medical Microbiology, Collage of Medicine, University of Wasit, Iraq.
[2]Al-Karama Teaching Hospital, Collage of Medicine, University of Wasit, Iraq.
* Corresponding author: marmar199265@gmail.com

Abstract

Coagulase-negative *Staphylococci* (Co.N.S) is opportunistic pathogens, widespread in clinical cases and contain many species. *Staphylococcus epidermidis* is a most frequent species of Co.N.S and have ability to produce biofilm making them difficult to eradicate. This study was intended to isolates of Co.N.S and to investigate the detection of biofilm forming isolates using traditional method (MTP) and molecular method (PCR) for most predominate species of Co.N.S. In addition, comprisal effect of some probiotic and some antibiotics on biofilm forming isolates. This study investigated 89 isolates of Co.N.S, which collected from different clinical source and the isolates identification, using (API Staph kit & Vitek2 system). Micro-titer plate method (MTP) used to detection of development biofilm. In addition, Molecular method (PCR) used to detection of some virulence gene (*Bap. icaA, icaD & FnbA*) that associated with biofilm forming. Used commercially available capsule (*L. acidophilus* & *L. rhamnosus*) as probiotic. Used well diffusion method to estimate activity of *Lactobacillus* spp. In addition, susceptibility testing was performed using 10 antibiotics. A total 89 (46.30%) isolates were identification Co.N.S. *S. epidermidis* 38(42.7%) was the most frequent species among Co.N.S isolates. A total (36) isolates of *S. epidermidis* were detected as *S. epidermidis* by molecular methods of 16SrRNA gene. The results of detection of formation biofilm showed 30(83.3%) isolates of *S. epidermidis* biofilm produce by MTP. The result of PCR to screening virulence genes in (30) isolates of *S. epidermidis* biofilm forming, demonstrated that 26 (86.7%) isolates harbored *icaA* and *icaD*, 29 (96.7%) isolates were harbored *FnbA,* and no-isolates have *bap* gene. The antibiotic test demonstrated a different percentage of resistance to each antibiotic. Whereas Vancomycin, Linezolid and Novobiocin non-showed resistance. On other hand, antimicrobial activity of *Lactobacillus* spp. (*L. rhamnosus* & *L. acidophilus*) against *S. epidermidis* showed highest inhibition zone recognized with bacterial culture broth (BCB) was with *L. rhamnosus* (17mm), and *L. acidophilus* (13mm) and the highest inhibition zone recognized with cell-free supernatant (CFS) was with *L. rhamnosus* (13mm), and *L. acidophilus* (11mm). In comprisal effect between some antibiotic and probiotic, showing some antibiotics such as (linezolid, vancomycin, Novobiocin) inhibited all biofilm forming *S. epidermidis* isolates, while *Lactobacillus* spp. cannot inhibited all biofilm-forming isolates. Non-of isolates harbored *bap* gene.

*Keywords***:** Co.N.S, *S. epidermidis*, biofilm, PCR, probiotic, antibiotics.

Introduction

Probiotic bacteria have beneficial effects on human health by improving the gut microbiota balance and inhibition pathogen (Heenan *et al*., 2014). It is well known that most probiotic is lactic acid bacteria (LAB), such as *Lactobacillus* species, *Enterococcus* species, *Bifidobacterium* species and some yeast (Ljungh and Wadström, 2012; Abdallah *et al*., 2013). These bacteria can secrete certain anti-microbial molecules, such as ethanol, fatty acid, hydrogen peroxide, and bacteriocins to exploit the antimicrobial activity (Georgieva *et al*., 2015; Inglin *et al*., 2015). There are a widespread distribution of coagulase-negative staphylococci (Co.N.S) on the human body ranging from normal flora to those that cause severe diseases (Gautam *et al*., 2017). About 65% to 90% of all Co.N.S isolated from human is *Staphylococcus epidermidis (S. epidermidis)* and it is the most clinically main species of Co.N.S in humans (Rogers *et al*., 2011). *S. epidermidis* is an important commensal organism of the human skin and mucous membranes (Nguyen *et al*., 2017) it is opportunistic pathogen has the ability to produce biofilms on surfaces of implanted tools, helping them to evade the innate immune system and protecting against antibiotics (Katherine *et al*., 2018). *S. epidermidis* is the major infection associated with the indwelling device; catheters related disease, surgical wounds, hospital-acquired infection, urinary tract infections and bacteremia (Mohammad *et al*., 2011; Upadhyayula *et al*., 2012). Biofilm formation is a four-stage process involving attachment, accumulation, maturation, and detachment, as well as *Bap*, *fbe* and *altE* genes are invented to play significant role in an initial attachment (Bowden *et al*., 2005). Polysaccharide intracellular adhesion (PIA) is an important virulence factor to form biofilm, it is encoded by several genes include *icaADBC* operon. *icaA* and *icaD* plays a central role in biofilm production in the accumulation stage (Arciola et *al*., 2002; Lou *et al*., 2011; Limoli *et al*., 2015). *FnbA* is a fibronectin-binding protein, promotes biofilm formation through binding of the protein to a surface-located receptor on adjacent cells (Tormo *et al*., 2005; Herman-Bausier *et al*., 2015).

Materials and Methods

Collection of sample and patients

A total 192 clinical samples were collected from different hospital and clinical private in Wasit province, during period December 2018 to April 2019. Patients were representing different age and gender. The characteristic of cases according to age found the mean of age was 35.744± 15.758 ranging from 5 years to 77 years, and the patient gender 125(65.1%) were female and 67(34.9%) were male.

Bacterial isolates and identification

All of 192 specimens culture on blood agar, mannitol salt agar and incubated at 37C° for 18-24 hours. The isolated bacteria Co.N.S were identified according to colony morphology, shape, size, color, biochemical test, API staph strip and Vitek2 System.

Biofilm detection

There are various methods to evaluate biofilm formation in bacteria. Micro-titer plates (MTP) is a best method used for detecting slime and analysis on quantitative (Christensen *et al.*, 1985; Abou El-Khier *et al.*, 2015).

Antibiotic susceptibility testing

Antibiotic sensitivity of isolates were identified on Mueller Hinton agar using Kirby-Bauer disk diffusion method as per CLSI guidelines (Sharma and Srivastava, 2016). Co.N.S species were considered resistance or sensitive based on zone of inhibition following the criteria of clinical and laboratory standard institute CLSI (CLSI, 2018). The antibiotics discs used in this study was Amikacin (30µg), Cefepime (30µg), Vancomycin (30µg), Nitrofurantoin (300µg), Ciprofloxacin (5µg), Piperacillin (100µg), Doxycycline (30µg), Meropenem (10µg), Linezolid (30µg), Levofloxacin (5 µg), Novobiocin (30µg).

Probiotic

Probiotic preparation

Probiotic strains from commercially available capsule *L. acidophilus* (Holland & Barrett, USA) and *L. rhamnosus* GG (Health Genesis, USA) were isolated by suspending in each capsule in 10 ml of MRS broth (Oxoid, UK) then incubated anaerobically at 37ºC for 18-24 hour (Forbes *et al.*, 2007). After preparation, probiotic used sterile cotton swabs were dipped into the cultures broth of *Lactobacillus* spp. and spread it on MRS agar (Oxoid, UK), then incubated anaerobically at 37°C for 24-48 hr.

- **Preparation of broth culture bacteria (BCB, *Lactobacillus* spp.) :** *Lactobacillus* spp. to be tested for antimicrobial activity were incubated in MRS broth (Oxoid, UK), anaerobically for 24 hours at 37°C. (Turbidity= 0.5 McFarland).

- **Preparation of cell-free supernatant (CFS, *Lactobacillus* spp.) :** Cell free supernatant (CFS) for each probiotic *Lactobacillus* spp. were produced using MRS broth (0.5 McFarland) incubated anaerobically for 18-20 hours at 37°C. Then, bacterial cells were removed by centrifuging the culture at 10000 rpm for 15 min at 4 °C. The pH values of supernatant were adjusted to pH 6.5 by the addition of 1N NaOH, the supernatant were membrane filtered (Millipore, 0.22µm) and stored at 4°C (Nowroozi, 2004).

- **Preparation of pathogenic bacteria :** Used nutrient broth (Oxoid, UK) to growing of pathogenic bacteria at 37 °C for 18-20 hours.

Antimicrobial activity using agar well diffusion method

The following method conducted as triplicate according to (Kalalou *et al.*, 2004). After 24 hours of incubation at 37°C, each plate was examined for the zone of inhibition the diameter of the inhibition zone was measured (in mm) with Vernier calipers.

DNA extraction and polymerase chain reaction (PCR) amplification

The DNA of isolates was extracted according to the instruction of Presto™ Mini gDNA bacteria Kit Quick protocol (Geneaid, USA). After DNA extraction, using Nano-drop (Eppendorf, Germany) to measure purity and concentration of DNA. The concentration was between (50-360 ng/µl), and the purity average (1.8-2). Standard PCR conventional using specific primers for 16S rRNA gene as shown in (Table 1) screened to all *S. epidermidis* isolates, and specific primers for detection of biofilm gene (*Bap*, *icaA*, *icaD* and *FnbA*) as shown in (Table 1) screened to all *S. epidermidis* isolates biofilm forming. PCR reaction tubes were transferred into thermal cycler (Bio-Rad, USA) that was programmed as shown in (Table 2).

Table 1: The specific primers and their sequence

Primer Name		Sequences 5'-3'	Size (bp.)	References
16S rRNA	F	TCAACCGTGGAGGGTCATTG	557bp	This study
	R	GTTTGTCACCGGCAGTCAAC		
icaD	F	CTATGCTACATGGTCAAGCC	245bp	This study
	R	ACAAACAAACTCATCCATCCG		
icaA	F	CTGTTTCATGGAAACTCC	200bp	Tremblay *et al.* (2013)
	R	TCGATGCGATTTGTTCAAACA		
bap	F	GGCGCAAGCAGCAGAATTA	901bp	Seng *et al.* (2017)
	R	CATAGTTCTTTGTGGTGTTGC		
FbnA	F	CCCTCTTCGTTATTCAGCC	422bp	Seng *et al.* (2017)
	R	CAGGAGGCAAGTCACCTTG		
	R	TTTGTAGATGTTGTGCCCCA		

Table 2: Thermal-cycling program for all gene

No.	Steps	Temperature °C	Time	Cycle
1	Initial Denaturation	95ºC	5 min.	1
2	Denaturation	95ºC	30 sec.	30
3	Annealing	59.3 ºC [1], 52.7 ºC [2] 56.5 ºC [3], 57.8ºC [4], 56.2 ºC [5]	30 sec.	
4	Extension	72ºC	50 sec.	
5	Final extension	72ºC	5 min.	1
6	Holding	4ºC	Forever	--

**1- 16S rRNA, 2- icaA, 3- icaD, 4- FnbA, 5- bap,

Statistical analysis

Data were coded and entered in the computer by using the statistic package ″SPSS· computer program. The results were expressed as mean ± Standard deviation (SD). Different groups were comparing by using student T test and the P value of < 0.05 were reflected statistically significant. This program SPSS version 25.0, Armonk, New York, 2015, Fishers exact test, chi-squared test (X^2 test).

Results

A total 89/192(46.3%) isolates identified Co.N.S. Eleven species belonged to Co.N.S were isolates and identified from various body sites (Table 3). *S. epidermidis* (42.7%) and *S. hominis* spp. *hominis* (18%) were the most commonly isolates Co.N.S species, followed by *S. xylosus* (11.2%), *S. lentus* (6.7%), *S. auricularis* (5.6%), *S. warneri* (4.5%) and *S. capitis* (3.4%). In present study, *S. cohnii* (2.2%), *S. lugdunensis* (2.2%), *S. sciuri* (2.2%), and *S. haemolyticus* (1.1%), had a lower rate of clinical isolation among Co.N.S species. *S. epidermidis* was the most frequent species in urine (21 isolates) followed by wound (9 isolates) then nasal (4 isolates).

Table 3 : The number and percentage of Co.N.S species from clinical source

Co.N.S species		Source of samples collections						
		Urine	Nasal	Catheter	Wound	Burns	Ear swab	Total
S. epidermidis	Count	21	4	2	9	1	1	38
	% of Total	23.6%	4.5%	2.2%	10.1%	1.1%	1.1%	42.7%
S. hominis spp. hominis	Count	10	2	3	1	0	0	16
	% of Total	11.2%	2.2%	3.4%	1.1%	0.0%	0.0%	18.0%
S. xylosus	Count	2	0	0	2	6	0	10
	% of Total	2.2%	0.0%	0.0%	2.2%	6.7%	0.0%	11.2%
S. lentus	Count	2	1	0	3	0	0	6
	% of Total	2.2%	1.1%	0.0%	3.4%	0.0%	0.0%	6.7%
S. cohnii	Count	2	0	0	0	0	0	2
	% of Total	2.2%	0.0%	0.0%	0.0%	0.0%	0.0%	2.2%
S. auricularis	Count	2	0	0	1	0	2	5
	% of Total	2.2%	0.0%	0.0%	1.1%	0.0%	2.2%	5.6%
S. warneri	Count	3	0	1	0	0	0	4
	% of Total	3.4%	0.0%	1.1%	0.0%	0.0%	0.0%	4.5%
S. capitis	Count	0	1	2	0	0	0	3
	% of Total	0.0%	1.1%	2.2%	0.0%	0.0%	0.0%	3.4%
S. sciuri	Count	0	0	2	0	0	0	2
	% of Total	0.0%	0.0%	2.2%	0.0%	0.0%	0.0%	2.2%
S. lugdunensis	Count	0	0	2	0	0	0	2
	% of Total	0.0%	0.0%	2.2%	0.0%	0.0%	0.0%	2.2%
S. haemolyticus	Count	0	0	0	1	0	0	1
	% of Total	0.0%	0.0%	0.0%	1.1%	0.0%	0.0%	1.1%
Total	Count	42	8	12	17	7	3	89
	% of Total	47.2%	9.0%	13.5%	19.1%	7.9%	3.4%	100.0%
P < 0.001								

Staphylococcus epidermidis

Molecular detection using PCR

In the present study, 38 isolates was identified *S. epidermidis* using API staph and Vitek-2 system, but 36 isolates were positive for the 16S rRNA and identified as *S. epidermidis*, Figure (1). In the current study, recorded 36 (100%) isolates was *S. epidermidis*.
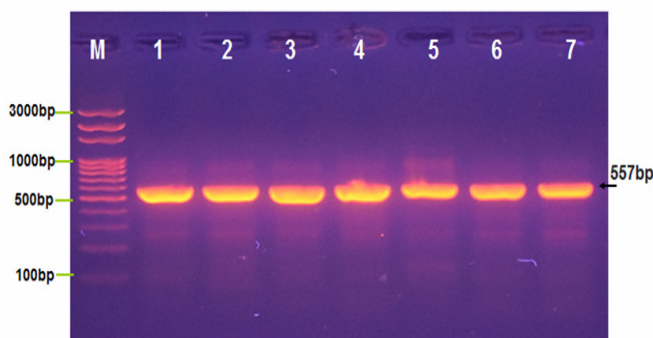

Fig. 1 : Agarose gel electrophoresis (1.5%) image that showed 16S rRNA gene in *S. epidermidis* isolates at 557bp PCR product size. Lane (M): DNA ladder (3000-100bp). Lane (1-7) positive for 16S rRNA. Lane (1, 7): Urine sample, Lane (2): Catheter sample, Lane (3): Wound sample, Lane (4): Nasal sample, Lane (5): Burn sample, Lane (6): Ear Sample.

Detection of biofilm formation

In present study, among 36 isolates of *S. epidermidis* 30(83.3%) isolate biofilm forming and 6 (16.7%) non-biofilm forming by MTP method. In addition, MTP method showed out of 30 isolates *S. epidermidis* biofilm production, 15 (50%) isolates was strong biofilm production, 9 (30%) isolate was moderate and 6 (20%) isolates was weak biofilm.

Molecular detection of biofilm gene using PCR

In present study, the detection of virulence genes (*icaA*, *icaD*, *bap* and *FnbA*) was done using PCR for 30 isolates of *S. epidermidis* biofilm forming, the results demonstrated that 26 (86.7%) were harbored *icaA* and *icaD*, 29 (96.7%) isolates

were harbored *FnbA*. Whereas no isolates have *bap* gene. Figure (2, 3, 4 and 5). In addition, in current study, four isolates (13.3%) were identified as biofilm producer by the phenotypic method but the *ica* gene was absent.
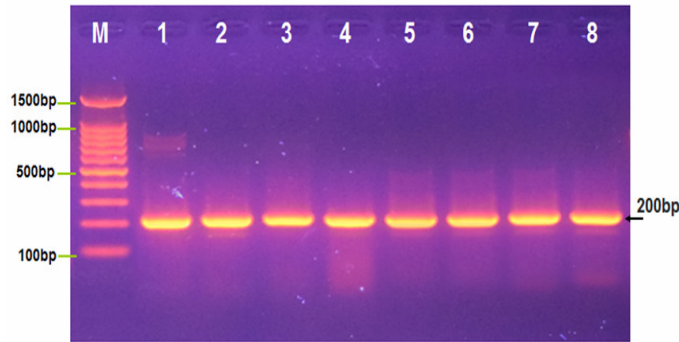

Fig. 2: Agarose gel electrophoresis (1.5%) image that showed *icaA* gene in *S. epidermidis* isolates at 200bp PCR product size. Lane (M): DNA ladder (1500-100bp). Lane (1, 7): urine sample, Lane (2, 8): Catheter-tip sample, Lane (3): wound sample, Lane (4): Nasal sample, Lane (5): Burn sample, Lane (6): Ear Sample.
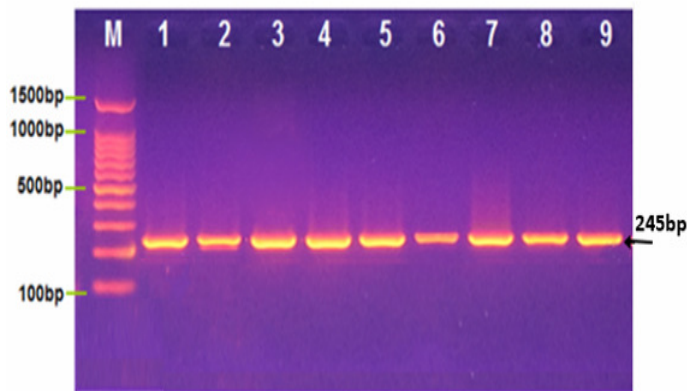

Fig. 3: Agarose gel electrophoresis (1.5%) image that showed *icaD* gene in *S. epidermidis* isolates at 245bp PCR product size. Lane (M): DNA ladder 3000-100bp). Lane (1, 7): urine sample, Lane (2, 8): Catheter-tip sample, Lane (3, 9): wound sample, Lane (4, 10): Nasal sample, Lane (5, 11): Burn sample, Lane (6, 12): Ear sample.
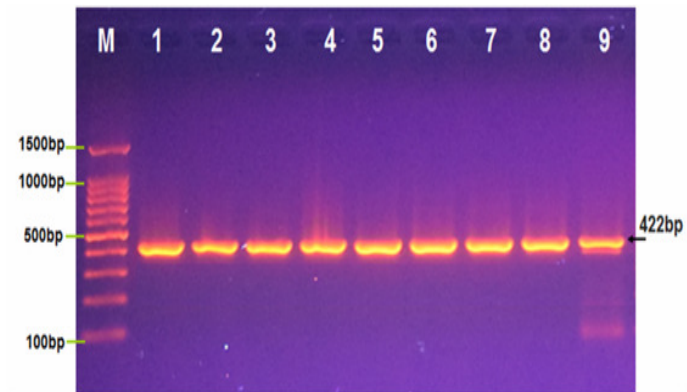

Fig. 4: Agarose gel electrophoresis (1.5%) image that showed *FnbA* gene in Staphylococcus epidermidis isolate at 422bp PCR product size. Lane (M): DNA ladder (1500-100bp). Lane (1, 7): urine sample, Lane (2, 8): Catheter-tip sample, Lane (3, 9): wound sample, Lane (4): Nasal sample, Lane (5): Burn sample, Lane (6): Ear Sample.
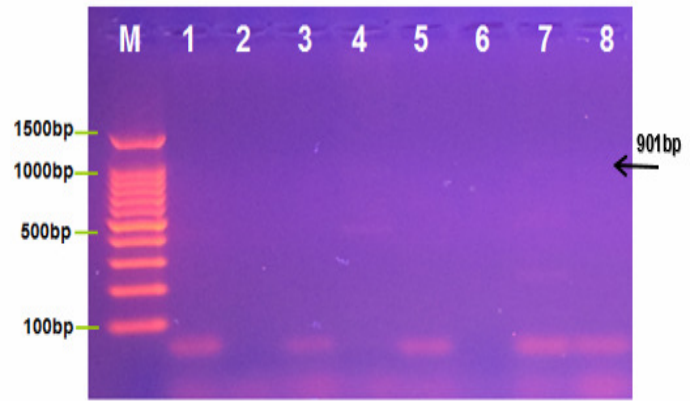

Fig. 5: Agarose gel electrophoresis (1.5%) image that showed negative *bap* gene in *S. epidermidis* isolate at 901bp PCR product size. Lane (M): DNA ladder (100-1500bp). Lane (1, 7): urine sample, Lane (2, 8): Catheter-tip sample, Lane (3): wound sample, Lane (4, 10): Nasal sample, Lane (5): Burn sample, Lane (6): Ear sample.

Antibiotic susceptibility test

The isolates of *S. epidermidis* showed different level of resistance to each antibiotic. The resistance percentage to cefepime, ciprofloxacin, doxycycline and levofloxacin were 61.1%, 58.3%, 44.4% and 41.7%, respectively. Moreover, the percentage of resistant to piperacillin 33.3%, amikacin 30.6%, and meropenem 13.9% and nitrofurantoin was 11.1%. In addition, isolates showed no resistance to linezolid and vancomycin (Table 4).

Table 4: Antibiotic susceptibility pattern of S. *epidermidis*

Antibiotics	S. epidermidis isolates=36					
	R		S		I	
	No.	%	No.	%	No.	%
Amikacin	11	30.6	25	69.4	--	--
Doxycycline	16	44.4	20	55.6	--	--
Nitrofurantoin	4	11.1	32	88.9	--	--
Levofloxacin	15	41.7	21	58.3	--	--
Meropenem	5	13.9	30	83.3	1	2.8
Ciprofloxacin	21	58.3	15	41.7	--	--
Cefepime	22	61.1	8	22.2	6	16.7
Piperacillin	12	33.3	18	50	6	16.7
Vancomycin	--	--	36	100	--	--
Linezolid	--	--	36	100	--	--
Novobiocin	--	--	36	100	--	--

Activity of *Lactobacillus* spp. (Probiotic)

Antimicrobial effect of *Lactobacillus* spp. against *S. epidermidis* was measured using well diffusion methods, this method were selected due to their ability to produce strong biofilms. The inhibition zone was measured by millimeters (mm). In present study, the highest inhibition zone with *L. rhamnosus* was 17 mm; while-highest inhibition zone with *L. acidophilus* was 13 mm using bacterial culture broth (BCB), (Table 5). In addition, the highest inhibition zone of bacterial cell free supernatant (CFS) in both *L. rhamnosus*, and *L. acidophilus* was (13 mm), and (11 mm), respectively, (Table 5). Based on the susceptibility tests, the effectiveness of *Lactobacillus* spp. isolates against bacterial pathogens was observed. Most of *S. epidemidis* isolates were sensitive to *Lactobacillus* spp. In current study, the probiotic *L. rhamnosus* has highest inhibition zones against *S. epidemidis* strains compared with *L. acidophilus*.

Table 5: Antimicrobial effect of *Lactobacillus* spp. in millimeters on biofilm produce *S. epidermidis.*

S. epidermidis strong biofilm N=15	*L. rhamnosus* activity (BCB)		*L. acidophilus* activity (BCB)	
	Inhibition zone 13-17 mm	No-inhibition zone	Inhibition zone 10-13 mm	No-inhibition zone
	11 (73.3%)	4 (26.7%)	11 (73.3%)	4 (26.7%)
S. epidermidis strong biofilm N=15	*L. rhamnosus* activity (CFS)		*L. acidophilus* activity (CFS)	
	Inhibition zone 11-13 mm	No-inhibition zone	Inhibition zone 9-11 mm	No-inhibition zone
	11 (73.3%)	4 (26.7%)	11 (73.3%)	4 (26.7%)

Discussion

The distribution species of Co.N.S varies in different samples depend on the type of cases. In present study, 89 (46.35%) isolates identified Co.N.S, many studies reported the same results (Muhammad *et al.*, 2014; Ibadin *et al.*, 2017). Meanwhile, the result disagreed with (Soumya *et al.*, 2017; Rashid and Omer, 2018). Differences in the percentage of infection in all our results and others may be due to different distribution of isolates that vary according to the clinical sampling area, environmental factors, nutrition requirements and virulence factors (Ogunseilan, 2005). In addition, the incompatible results of all these studies may be due to differences in the number of clinical isolates from a different region or because of isolates from patients with different clinical and physiological conditions (Lomholt *et al.*, 2001). In current study *S. epidermidis* (42.7%) was the most commonly isolates Co.N.S species. This result agreed with (Asaad *et al.*, 2016; Vale *et al.*, 2019). The finding of *S. epidermidis* as the most commonly isolated species in present study, might be because this organism produces biofilm, which helps it to attach to surfaces, and it is the most prevalent bacterium in human skin and mucosa (Kalalou *et al.,* 2004; Ogunseilan, 2005)*. S. epidermidis* is now recognized as an important opportunistic pathogen. This microorganism is one of the most communal causative agents of medical device-related infections (Sievert *et al.*, 2014). The ability to adhere to the surfaces of biomaterials, and subsequently form a biofilm is one of the most important factors for *S. epidermidis* to induce serious nosocomial infections (Mathur *et al.*, 2006; Liberto *et al.*, 2009). MTP method showed out of 30 isolates *S. epidermidis* biofilm production, 15 (50%) isolates was strong biofilm production, 9 (30%) isolate was moderate and 6 (20%) isolates was weak biofilm, the result is agreed with (Solati *et al.*, 2015). Biofilm detection using MTP method is more sensitive and helps in qualitative assessment of biofilm formation. Deka, (2014) that suggested the MTP method is a quantitative gold standard method for detection of biofilm formation, also supports the finding. *S. epidermidis* isolates obtained from clinical samples can ability to produce biofilm, and formation biofilm should be measured by any methods in routine microbiology laboratories. The result of *Bap* gene in current study, accordance with (Barbieri *et al.*, 2015; Salgueiro *et al.*, 2017), while Harris *et al.* (2016) found 20% of isolates carry *Bap* gene. Our result support the suggestion of other author, that the role of *Bap* gene in human infection is doubtful (Rohde *et al.*, 2007). The result of *icaA* and *icaD*, in present study comparable with (Mirzaei *et al.*, 2017; Salgueiro *et al.*, 2017). In addition, in current study, four isolates (13.3%) were identified as biofilm producer by the phenotypic method but the *ica* gene was absent. PIA production is not always correlated with biofilm formation, in recent studies; it

has become clear that there are at least two mechanisms of biofilm development. Mechanism requires the production PIA by the *ica* operon and other *ica*-independent mechanisms described biofilm production in absence of PIA as process (Seidl *et al.*, 2008; Lauderdale *et al.*, 2009). In an effort to explain these un-expected findings, some investigators reported the presence of certain genes in *ica*-negative biofilm-forming staphylococci, the accumulation-associated protein (*aap*) (Rohde *et al.*, 2005); this gene was found to induce an alternative PIA-independent mechanism of biofilm formation. The detection of *FnbA* gene using PCR revealed 29 (96.7%) isolates carry this gene. *FnbA* has been reported in a high quantity among the staphylococcal strains is dated form various clinical infections (high than 95%). Also, Bartoszewicz *et al.* (2004) is suggesting that the absence of *ica* gene this may be due to an *ica* gene independent control of biofilm formation/adhesion process in staphylococci. The difference in antimicrobial activity of different *Lactobacillus* spp. via production of numerous substances, such as fatty-acid, hydrogen-peroxide, ethanol, and bacteriocins to exploit the anti-microbial activity (Georgieva *et al.*, 2015; Inglin *et al.*, 205). Also, production and release of antimicrobial molecules by *Lactobacillus* spp. is known to be variable with factor such as cell density and population kinetics (Delgado *et al.*, 2007; Holzapfel and Wood, 2012). The antimicrobial effect of BCB using well diffusion method observed highest inhibitory zones in the diameters compared to CFS. This may be due to presence of greater levels of inhibitory substances in the (BCB), such as those associated with whole bacteria and antimicrobial substances, also to the presence of other secondary metabolites by *Lactobacillus* spp. such as the LAs, bio surfactant, and other fermentation product, as well as bacteriocins may play an important role (Jamalifar *et al.*, 2011). The present study agrees with some in *vitro* studies, which have also shown that *Lactobacillus* strains can exhibit antimicrobial activity against *C. difficile*, *E. coli*, *Shigella* spp., *S. mutans*, *P. aeruginosa,* and *S. aureus* (Mirnejad *et al.*, 2013; Kumar *et al.*, 2016; Kang *et al.*, 2017; Ahn *et al.*, 2018).

Conclusion

In the comparison effect between probiotic and some antibiotics, in present study, none of the *Lactobacillus* spp. was effective against all the strong biofilm producing strains, but *S. epidermidis* isolates showed fully sensitive to the some antibiotics such as, Novobiocin, vancomycin and linezolid. Furthermore, isolates showed a good susceptibility to other antibiotics such as, nitrofurantoin and meropenem. MTP is best method to detection formation biofilm. None of isolates have *Bab* gene, it is doubtful in human infection.

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