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Antimicrobial Activity of Non-bond Colicin on *Candida albicans* Biofilm

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Abstract

Two hundred fifty mid-stream urine specimens were collected from Baqubah Teaching Hospital and Al-Batool Teaching Hospital from patients with urinary tract infections (UTI). Of these investigated urine specimens, 66 (26.4%) specimens showed positive growth culture of Gram-negative bacteria. From these, *Escherichia coli* was the most prevalent bacteria of the examined culture (41, 62.12%). Additionally, the cup assay was used to determine colicin producers while the most efficient colicin producers were estimated by the formation of larger inhibition zone. Approximately half of the investigated *E. coli* isolates (20, 49 %) was colicin producers. Colicins was extracted after induction by mitomycin-C showed a concentration of 3020 µg/ml, as estimated utilizing the Lowry method, while its activity was 80 U/ml. Our study results showed that colicin had significant antibiofilm activity ($P \leq 0.05$) against *Candida albicans* and the effect seemed to be concentration dependent. . However, the values of biofilm inhibition varied depending on the different tested isolates. The biofilm of isolate 5 showed the most significant inhibition ($P \leq 0.05$) by colicin with a value of 46%, while isolate 3 was less affected with an inhibition rate of 19% at the concentration of 2500 µl/ml.

Keywords: *Escherichia coli*, Colicin, *Candida albicans*, Antibiofilm.

الفعالية الضد مايكروبية للكولسين الغير مرتبط على الاغشية الحيوية للمبيضات البيضاء

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الخلاصة

تم عزل 250 عينة ادرار من الاشخاص المصابين بالتهاب المجاري البولية من مستشفى بعقوبة التعليمي و مستشفى البتول التعليمي. وجدت ان هنالك 66 عينة ادرار(26.40%) من 250 عينة لديها نمو بكتيري للعزلات السالبة لصبغة كرام. الاشريكية القولونية كانت اكثر العزلات انتشارا مقارنة بالعزلات الاخرى, حوالي 41 عزلة (62.12%) من العزلات السالبة لصبغة كرام. تم التحري عن الاشريكية القولونية المنتجة للكوليسين بأستخدام طريقة اقراص الآكار حيث تم اختيار العزلة الاكثر كفاءة لانتاج الكوليسين من خلال قياس قطر منطقة تثبيط النمو. وجد ان العزلات المنتجة للكوليسين كانت اقل بقليل من العزلات الغير منتجة له (اي بنسبة49 %). بعد عملية استخلاص الكوليسين تم تحديد تركيز البروتين للكولسيين و بقيمة 3020 مايكروغرام/ مل باستخدام طريقة لاوري و كذلك تم تقدير فعالية الكوليسين و بقيمة 80 وحدة/مل باستخدام طريقة الحفر. من خلال هذه الدراسة وجد ان للكولسين الخام له تأثير مثبط لتكوين البايوفلم المنتج من قبل

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المبيضات الفطرية وبصورة معنوية($P\leq 0.05$). لوحظ انه كلما ازداد تركيز الكولسين كلما زاد معه التأثير المثبط للخميرة معنويا ($P\leq 0.05$) اي ان العلاقة طردية مابين نسبة التثبيط والتركيز المستخدم. فضلا" عن ذلك فأن النسبة المئوية لتثبيط البايوفلم تختلف باختلاف عزلات الخمائر المستخدمة في التجربة وبصورة معنوية عند مستوى احتمالية (5%), إذ ان العزلة رقم (5) كانت اكثر العزلات تحسسا" للكوليسين و بنسبة تثبيط بلغت 46% مقارنة بمجموعة السيطرة , في حين العزلة رقم (3) كانت الأكثر مقاومة وبصورة معنوية ($P\leq 0.05$)، فقد سجلت نسبة تثبيط مقدارها (19%)عند التركيز 2500مايكروغرام/مل.

Introduction

Escherichia coli is one of the most common pathogens, which causes a wide spectrum of diseases within and outside the intestinal tract[1]. Extraintestinal pathogenic *E. coli* (ExPEC) is the main causative agent of urinary tract infection, enteritis, septicemia and other infections such as neonatal meningitis [2]. One of its key pathogenicity features is the production of bacteriocins[3]. Bacteriocins are ribosomal synthesized antimicrobial peptides that have the ability to kill or inhibit the growth of other strains, without damaging the producing bacteria due to having specific immunity proteins [4]. These peptides are different in many features like molecular mass, the existence of post-translational modifications, mechanisms of bacteriocins release from producer cells, and others [5]. Genes of bacteriocin biosynthesis are clustered and encoded on plasmids, chromosome and/or transposons [6]. It is believed that the killing mechanism of colicins produced by *E. coli* can be accomplished by pore formation in the inner membrane of the target cell and degradation of intracellular components such as DNA and RNA [7].

Candida albicans is a dimorphic fungus that may be found as commensal in the oral cavity of healthy people, but it also causes recurrent, severe and even lethal systemic infections [8]. It is thought that the rising rate of immunocompromised patients could lead to increase the risk of candidiasis [9]. C. *albicans* can infect skin, mouth, throat and blood [10]. This may be attributed to the possession of many virulence factors that help C. *albicans* to infect the host. C. *albicans* virulence factors include polymorphism, adhesins and invasions, hydrolases, germ tube formation and biofilm [11]. Biofilm is a population of microorganisms attached to the solid surfaces and embedded in extracellular polymeric substances (EPS) that are composed of proteins, carbohydrates, and nucleic acids. Microorganisms usually produce these EPS matrix in a complex structure, which is comparable to honeycombs of the hive, that supports them as a mechanical defense and resistance against antimicrobials [12]. Patients can acquire infection due to the presence of biofilms on hospital equipment and medical devices, eventually leading to persistent infections [13].

There are several reasons to analyze the effect of colicin as antimicrobial against candida, such as the appearance of antimicrobial resistance candida and the side effects of these drugs as well as the spread of drug resistant biofilms.

Materials and methods

Isolation and identification of bacterial and fungal isolates

Mid-stream urine specimens were collected from Baqubah Teaching Hospital and Al-Batool Teaching Hospital from patients clinically diagnosed with urinary tract infections (UTI). The isolates were identified utilizing microscopic examination. Morphological features of the colonies and biochemical tests were conducted according to Brenner and Farmer[14] as well as by using chrome agar and Vitek-2 system.

C. albicans isolates were obtained from oral swabs from patients with renal impairment. *C. albicans* isolates detection was confirmed by forming germ tubes [15] and by Vitek-2 system.

Detection of colicin-producing isolates

Colicin-producing *E. coli* were detected using cup assay[16]. The most efficient producers showed the largest inhibition zone and the feature of the stability of bacteriocin production.

Extraction of crude non-bound colicin.

Previously incubated 2.5ml of nutrient broth with the selected colicin producer were added to sterile nutrient broth supplied with 5% of glycerol and then incubated for 14h at 37 ℃. After the addition of 2μg/ml of mitomycin-C, they were incubated in an incubator shaker for 3 hrs then centrifuged at 5000 rpm for 30 min using refrigerated centrifuge. The non-bound colicin in the supernatant was separated from the cells. To eradicate the remaining cells, chloroform was added. To confirm the bacterial clearance, the supernatant was cultured on brain heart infusion. The activity of

colicin was detected by using the well method [17] and colicin concentration was estimated by Lowry method [18,19].

Biofilm formation of *C. albicans*

After incubation of *C. albicans* isolates on sabouraud dextrose broth, they were diluted by sterile broth at the ratio of 1:20. Each well of the 96-well flat microtiter plates were filled with 200 µl of these isolate suspensions. Sabouraud dextrose broth was also used as negative control in separate wells of the 96-well flat microtiter plate. Experiments were performed in triplicates in which the plates were incubated for 48hrs at 37 ℃. Following the incubation, the medium and the unbound cells were removed; the wells were washed by Phosphate Buffer Saline (PBS) then left to dry for 15 mins. 200 µl of Crystal Violet (CV) were added to the wells and left for 20 mins. CV was removed and the plates were washed by PBS three times then left to dry at room temperature. 200 µl of solution of acetone: ethanol (20:80) was added to each well and left for 10 min. Plate reader apparatus (Biotek/ USA) was used for reading the results at 450 nm. The optical density (OD) values were estimated, where the values > 0.320, $0.120 - 0.320$, and < 0.120 were considered as reflecting strong, moderate, and weak reactions, respectively [20].

The inhibition of *C. albicans* biofilm formation

The suspensions of *C. albicans* were inoculated to 96- flat well microtiter plates previously inoculated with colicin at Minimum Inhibitory Concentration (MIC) concentrations. After 48hrs of incubation, the wells were washed by PBS then left to dry. 200 µl of CV was added for 20 min. CV was removed and the plates were washed by PBS and left to dry. 200 µl of acetone: ethanol solution (20:80) was added to the wells and left for 20 min, then the result was read by the plate reader [21, 22]. Biofilm inhibition was calculated using the below equation [23]:

$$\text{Inhibition of biofilm formation\%} = \frac{\text{OD control} - \text{OD treatment}}{\text{OD control}} \times 100$$

Results and discussion

Isolation and identification

Out of 250 urine samples, 66 (26.4%) isolates had bacterial growth, as shown in Figure-1. *E. coli* was the most prevalent Gram-negative bacteria in UTI samples, which was recorded in 40 (60.60%) of the 66 Gram-negative isolates (Figure-2).

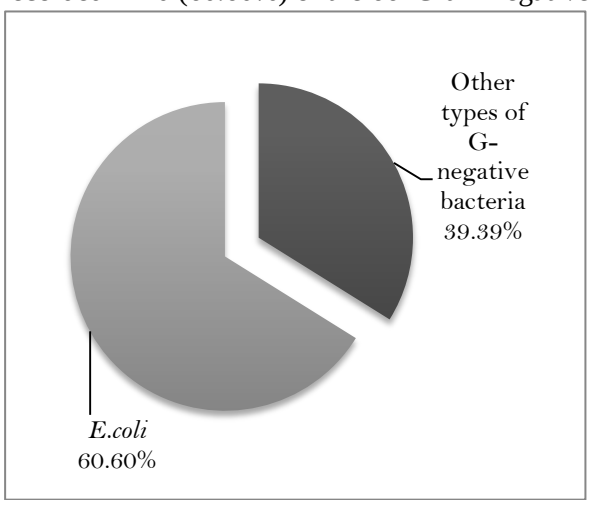

Figure 1-The percentage of bacterial growth with positive cultures from UTI isolated from UTI patients.

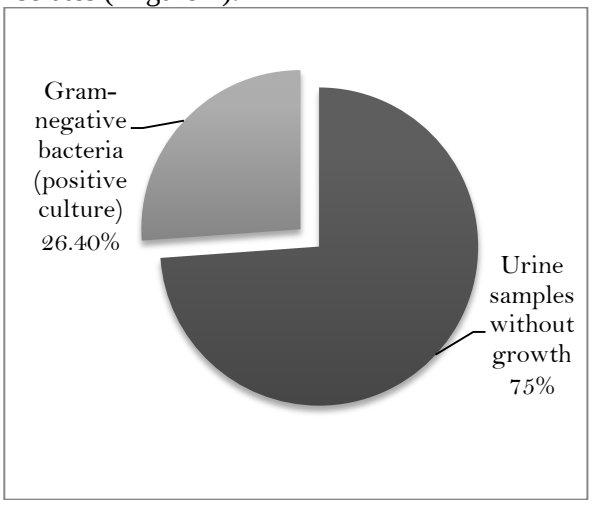

Figure 2-The percentage of *E. coli* isolates among other G- bacteria urine specimens

In this respect, a previous study reported that the prevalence of *E. coli* (38.90%) was higher than the other bacteria in UTI cases [24]. In this regard, several conditions may affect the prevalence of bacteria among patients. These may involve environmental, health, social, and cultural conditions of patients. In addition, the technical mistakes for isolation and identification of bacteria may give inconsistency in the reported findings[25].

Detection of colicin-producing *E. coli* by cup assay

By the detection of the inhibition zone using cup assay, we observed that 20 *E. coli* isolates (49%) were colicin producers (Figure-3) and the most effective isolate had the larger inhibition zone.

However, this result seems relatively different from the findings of a recent research [26] which illustrated that *E.coli* was the most colicin producing bacterial isolate which was produced by 36 (30.77%) out of 117 *E.coli* isolates. These differences occur due to several reasons such as the components of the used media [27] and the different methods used for the detection of colicin production [28]. Another study reported that the use of the cup assay and the addition of 5% glycerol gives robust results for the detection of bacteriocin producers [29].

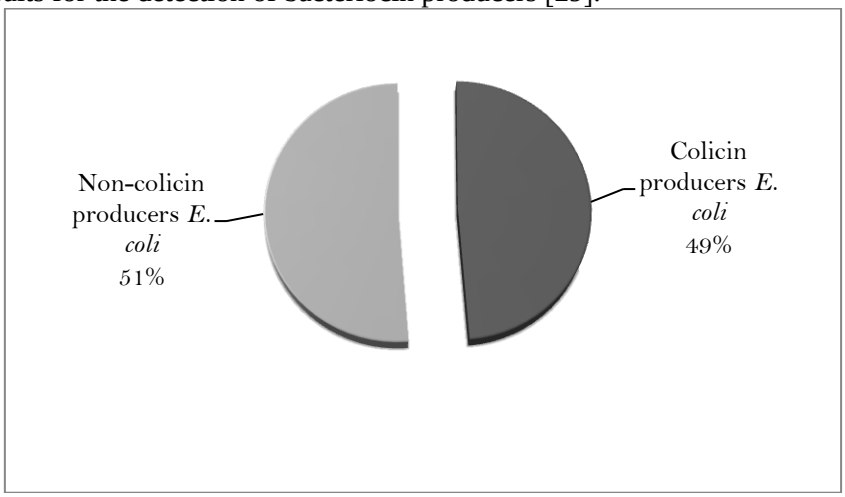

Figure 3-Percentage of cup assay of colicin producing *E. coli* isolated from UTI patients.

Biofilm formation of *C. albicans*

Biofilm formation of *C.albicans* was assessed by the microtiter plate method [20]. The isolates 2 and 5 were found to form strong biofilms and their optical density values were 0.36 and 0.337, respectively, while moderate biofilms were found to be formed by the others isolates, with optical density ranged from 0.249 for isolate 1 to 0.313 for isolate 7 (Table-1).

Table 1-Values of Tissue Culture Plate (TCP) method of biofilm formation *of C. albicans* isolated from oral swabs of patients with renal impairment

***C. albicans* isolates**	**OD**	**Estimation of biofilm formation**
C1	0.249	Moderate
C2	0.36	Strong
C3	0.269	Moderate
C4	0.278	Moderate
C5	0.337	Strong
C6	0.276	Moderate
C7	0.313	Moderate
C8	0.312	Moderate

An earlier study showed that *C. albicans* colonizing the oral cavity can form a biofilm on saliva coating areas [30]. Consistence with our finding, Udayalaxmi and Shenoy reported that 45.83% of Candida species were strong or moderate biofilm formers while the percentage of isolates that produce weak biofilm in their study was 54.16% [31].

Inhibition of biofilm formation by non-bond colicin

The results of biofilm inhibition were dependent on the concentration of colicin and the type of indicator *E. coli* isolate. This was evident when the higher concentrations of colicin led to significantly ($P \leq 0.05$)increased inhibition of biofilm; . Thus, the relation between the extracted colicin concentration and the biofilm inhibition is inversely proportional in the tested *C. albicans isolates* .

Table 2-Inhibition of biofilm formation by non-bond colicin extracted from *E. coli* isolated from UTI patients.

Concentration of colicin µg/ml	Inhibition of biofilm formation %								LSD value
	C1	C2	C3	C4	C5	C6	C7	C8	
19.53	20	3	3	1	21	12	6	6	5.72*
39.06	30	3	5	7	27	20	6	6	5.36*
78.125	33	3	5	13	34	22	7	9	5.09*
156.25	33	3	7	16	35	22	9	16	4.66*
312.5	35	18	8	17	36	25	18	19	5.38*
625	39	21	10	19	36	29	23	20	6.01*
1250	39	29	16	30	43	33	29	21	5.82*
2500	43	31	19	39	46	39	39	33	6.39*
LSD value	6.42 *	5.77 *	5.61 *	6.03 *	7.29 *	7.53 *	6.21*	6.64 *	---

* ($P<0.05$).

The results demonstrated in Table- 2 show that all *C. albicans* isolates were inhibited significantly ($P\leq 0.05$) by colicin, but their sensitivity was variant depending on the isolates. The isolates 1 and 5 were the most sensitive to colicin at the concentration 2500 µg/ml, with inhibition of biofilm values of 43% and 46%, respectively. At the same concentration, isolate 3 was the less sensitive isolate that showed a value of biofilm inhibition of 19%. There are some explanations of colicin action on the biofilm of microorganisms. Some bacteriocins act by disrupting the co-aggregation process of the membranes which is important for biofilm stability; thus it decreases biofilm development by reducing its biomass and thickness [32].

Other bacteriocins cause pore formation that results in an efflux of ATP from biofilm cells. The size of pores has to be larger than 1.5 in diameters which is enough to cause efflux of ATP [33]. Moreover , some bacteriocins have the ability to suppress biofilm genes such as *atl* (autolysin) and *ica* (intercellular adhesin) such as bacteriocin gallidermin [34].

Furthermore, ColA-43862 produced by *Citrobacter freundii* is known to have anti-biofilm activity, but it may not act as a limiting factor due to the complication of biofilm and its microenvironment that act as a barrier of colicins action [35].

An earlier study reported that a bacteriocin of *Lactobacillus acidophilus* had remarkably reduced biofilm cells of catheter-associated multidrug-resistance *Pseudomonas aeruginosa.* This bacteriocin can act as an alternative for antibiotics that hardly eliminate biofilm of *P. aeruginosa* [36]. A recent study reported that a bacteriocin of *Bacillus subtilis* (subtilocin) caused biofilm inhibition of *Gardnerella vaginalis*, with an inhibition value higher than 90%, however, it did not decrease the growth of planktonic cells. Also, it remarkably inhibited the biofilm of *E. coli* and *L. monocytogenes*. Inhibition of biofilm by these bacteria occurs because of their ability to inhibit the quorum sensing (QS) [37]. Bacteriocin EntV of *Enterococcus faecalis* has a reduction activity on virulence factors of *C. albicans* without affecting the viability of cells. It blocks hypha formation, which results in preventing biofilm formation as well as reducing inflammation and invasion of the epithelium by candida in marine models [38].

Conclusions

Biofilm of *C. albicans* was significantly inhibited ($P\leq 0.05$) by crude non-bond colicin and the inhibition effect was more evident at high concentrations of the extracted colicin. However, the biofilm inhibition effect of the *E. coli* extracted colicin seemed to be *C. albicans* isolates-specific as some isolates were more sensitive to colicin while others were less affected.

References

1. Jaureguy, F., Landraud, L., Passet, V., Diancourt, L., Frapy, E., Guigon, G., Carbonnelle, E., Lortholary, O., Clermont, O., Denamur, E. and Picard, B. **2008**. Phylogenetic and genomic diversity of human bacteremic *Escherichia coli* strains. *BMC genomics*, **9**(1): 560. doi: 10.1186/1471-2164-9-560.

2. Allocati, N., Masulli, M., Alexeyev, M.F. and Di Ilio, C. **2013**. *Escherichia coli* in Europe: an overview. *International journal of environmental research and public health*, **10**(12): 6235-6254. doi: 10.3390/ijerph10126235.

3. Budič, M., Rijavec, M., Petkovšek, Ž. and Žgur-Bertok, D. **2011**. *Escherichia coli* bacteriocins: antimicrobial efficacy and prevalence among isolates from patients with bacteraemia. *PLoS One*, **6**(12): e28769. doi: 10.1371/journal.pone.0028769.

4. Azpiroz, M.F., Poey, M.E. and Laviña, M. **2009**. Microcins and urovirulence in *Escherichia coli*. *Microbial pathogenesis*, **47**(5): 274-280. doi: 10.1016/j.micpath.2009.09.003.

5. Silva, C.C., Silva, S.P. and Ribeiro, S.C. **2018**. Application of bacteriocins and protective cultures in dairy food preservation. *Frontiers in microbiology*, **9**(1): 594. doi: 10.3389/fmicb.2018.00594.

6. Klaenhammer, T.R. **1993**. Genetics of bacteriocins produced by lactic acid bacteria. *FEMS microbiology reviews*, **12**(1-3):39-85. DOI: 10.1111/j.1574-6976.1993.tb00012.x.

7. Jin, X., Kightlinger, W., Kwon, Y.C. and Hong, S.H. **2018**. Rapid production and characterization of antimicrobial colicins using *Escherichia coli*-based cell-free protein synthesis. *Synthetic Biology*, **3**(1): ysy004. https://doi.org/10.1093/synbio/ysy004.

8. Noble, S.M., Gianetti, B.A. and Witchley, J.N. **2017**. *Candida albicans* cell-type switching and functional plasticity in the mammalian host. *Nature Reviews Microbiology*, **15**(2): 96. doi: 10.1038/nrmicro.2016.157.

9. Sellam, A. and Whiteway, M. **2016**. Recent advances on *Candida albicans* biology and virulence. *F1000Research*, **5**: 2582. doi: 10.12688/f1000research.9617.1.

10. Mayer, F.L., Wilson, D. and Hube, B. **2013**. *Candida albicans* pathogenicity mechanisms. *Virulence*, **4**(2): 119-128. doi: 10.4161/viru.22913.

11. De Souza, P.R., De Andrade, D., Cabral, D.B. and Watanabe, E. **2014**. Endotracheal tube biofilm and ventilator-associated pneumonia with mechanical ventilation. *Microscopy research and technique*, **77**(4): 305-312. doi: 10.1002/jemt.22344.

12. Hall, C.W. and Mah, T.F. **2017**. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS microbiology reviews*, **41**(3):276-301. doi: 10.1093/femsre/fux010.

13. Garsin, D.A. and Lorenz, M.C. **2013**. *Candida albicans* and *Enterococcus faecalis* in the gut: synergy in commensalism?. *Gut microbes*, **4**(5):409-415. doi: 10.4161/gmic.26040.

14. **14-**Brenner, D.J. and Farmer III J. J. **2005**. Family I. Enterobacteriaceae. In: Brenner, D. J., Krieg, N. R., and (eds)**,** *Bergey's Manual of Systematic Bacteriology*, 2nd edition. New York: Springer. 587—607.

15. Matare, T., Nziramasanga, P., Gwanzura, L. and Robertson, V. **2017**. Experimental Germ Tube Induction in *Candida albicans*: An Evaluation of the Effect of Sodium Bicarbonate on Morphogenesis and Comparison with Pooled Human Serum. *BioMed research international*, 2017. doi: 10.1155/2017/1976273.

16. Al-Qassab, A.O. and Al-Khafaji, Z.M. **1992**. Effect of different conditions on inhibition activity of enteric lactobacilli against diarrhea-causing enteric bacteria. *Journal of Agricultural Science*, **3**(1): 18-26.

17. Herschman, H.R. and Helinski, D.R. **1967**. Purification and characterization of colicin E2 and colicin E3. *Journal of Biological Chemistry*, **242**(22): 5360-5368. http://www.jbc.org/content/242/22/5360**.**

18. Smajs, D., Pilsl, H. and Braun, V. **1997**. Colicin U, a novel colicin produced by *Shigella boydii*. *Journal of bacteriology*, **179**(15): 4919-4928. doi: 10.1128/jb.179.15.4919-4928.1997**.**

19. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. **1951**. Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*, **193**: 265-275. https://www.ncbi.nlm.nih.gov/pubmed/14907713.

20. Deepa, K., Jeevitha, T. and Michael, A. **2015**. In vitro evaluation of virulence factors of Candida species isolated from oral cavity. *Journal of Microbiology and antimicrobials*, **7**(3):28-32. https://doi.org/10.5897/JMA2015.0337.

21. Dhanasekaran, D., Vinothini, K., Latha, S., Thajuddin, N. and Panneerselvam, A. **2014**. Human dental biofilm: Screening, characterization, in vitro biofilm formation and antifungal resistance of Candida spp. *The Saudi Journal for Dental Research*, **5**(1): 55-70. https://doi.org/10.1016/j.ksujds.2013.10.001.

22. Ali, O.A.U. **2012**. Prevention of *Proteus mirabilis* biofilm by surfactant solution. *Egypt Academic Journal of Biological Science*, **4**:1-8. http://microbiology.eajbs.eg.net/pdf/vol4.n1/1.pdf.

23. Chevalier, M., Medioni, E. and Prêcheur, I. **2012**. Inhibition of *Candida albicans* yeast–hyphal transition and biofilm formation by Solidago virgaurea water extracts. *Journal of medical microbiology*, **61**(7):1016-1022. doi: 10.1099/jmm.0.041699-0.

24. Majeed, H.T. and Aljanaby, A.A.J. **2019**. Antibiotic Susceptibility Patterns and Prevalence of Some Extended Spectrum Beta-Lactamases Genes in Gram-Negative Bacteria Isolated from Patients Infected with Urinary Tract Infections in Al-Najaf City, Iraq.*Avicenna journal of medical biotechnology*, **11**(2):192. https://europepmc.org/article/med/31057723.

25. Salman, S.B. and Al-Mathkhury, H.J.F. **2016**. Molecular Detection of *Klebsiella pneumoniae* serotype K2 Isolated Clinically. *Iraqi Journal of Science*, **57**(1A):98-103. https://pdfs.semanticscholar.org/3cec/12808875845b27433963922156b14e588be1.pdf.

26. Obaid, H.H. **2016**. Detection of Bacteriocin Production and Induction by Brassica rapa extract. *Ibn AL-Haitham Journal for Pure and Applied Science*, **29**(3):234-248. http://ircoedu.uobaghdad.edu.iq/jih/index.php/j/article/view/714.

27. Richardson, H., Emslie-Smith, A.H. and Senior, B.W. **1968**. Agar diffusion method for the assay of colicins. *Applied and Environmental Microbiology*, **16**(10):1468-1474. https://aem.asm.org/content/16/10/1468.

28. Özdemir, G.B., Oryaşın, E., Bıyık, H.H., Özteber, M. and Bozdoğan, B. **2011**. Phenotypic and genotypic characterization of bacteriocins in enterococcal isolates of different sources. *Indian journal of microbiology*, **51**(2):182-187. doi: 10.1007/s12088-011-0143-0.

29. Al-Charrakh, A.H., Yousif, S.Y. and Al-Janabi, H.S. **2011**. Antimicrobial spectrum of the action of bacteriocins from Klebsiella isolates from Hilla/Iraq. *Iranian Journal of Microbiol*ogy, **2**(5):76-78. https://www.iasj.net/iasj?func=fulltext&aId=140076.

30. Bamford, C.V., d'Mello, A., Nobbs, A.H., Dutton, L.C., Vickerman, M.M. and Jenkinson, H.F. **2009**. *Streptococcus gordonii* modulates *Candida albicans* biofilm formation through intergeneric communication. *Infection and immunity*, **77**(9):3696-3704. doi: 10.1128/IAI.00438-09.

31. Udayalaxmi, J. and Shenoy, N. **2016**. Comparison between biofilm production, phospholipase and haemolytic activity of different species of Candida isolated from dental caries lesions in children. *Journal of clinical and diagnostic research,***10**(4):DC21. doi: 10.7860/JCDR/2016/17019.7643.

32. Shin, J.M., Ateia, I., Paulus, J.R., Liu, H., Fenno, J.C., Rickard, A.H. and Kapila, Y.L. **2015**. Antimicrobial nisin acts against saliva derived multi-species biofilms without cytotoxicity to human oral cells, *Frontiers in microbiology*, **6**:617. doi: 10.3389/fmicb.2015.00617.

33. McAuliffe, O., Ryan, M.P., Ross, R.P., Hill, C., Breeuwer, P. and Abee, T. **1998**. Lacticin 3147, a broad-spectrum bacteriocin which selectively dissipates the membrane potential. *Applied and Environmental Microbiology*, *64*(2):439-445. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC106063/.

34. Zoll, S., Schlag, M., Shkumatov, A.V., Rautenberg, M., Svergun, D.I., Götz, F. and Stehle, T. **2012**. Ligand-binding properties and conformational dynamics of autolysin repeat domains in staphylococcal cell wall recognition. *Journal of bacteriology*, **194**(15):3789-3802. doi: 10.1128/JB.00331-12.

35. Shanks, R.M., Dashiff, A., Alster, J.S. and Kadouri, D.E. **2012**. Isolation and identification of a bacteriocin with antibacterial and antibiofilm activity from *Citrobacter freundii*. *Archives of microbiology*, **194**(7):575-587. doi: 10.1007/s00203-012-0793-2.

36. Al-Mathkhury, H.J.F., Ali, A.S. and Ghafil, J.A. **2011**. Antagonistic effect of bacteriocin against urinary catheter associated *Pseudomonas aeruginosa* biofilm. *North American journal of medical sciences*, **3**(8):367. doi.org/10.4297%2Fnajms.2011.3367

37. Algburi, A., Zehm, S., Netrebov, V., Bren, A.B., Chistyakov, V. and Chikindas, M.L. **2017**. Subtilosin prevents biofilm formation by inhibiting bacterial quorum sensing. *Probiotics and antimicrobial proteins*, **9**(1):81-90. doi: 10.1007/s12602-016-9242-x.

38. Graham, C.E., Cruz, M.R., Garsin, D.A. and Lorenz, M.C. **2017**. *Enterococcus faecalis* bacteriocin EntV inhibits hyphal morphogenesis, biofilm formation, and virulence of *Candida albicans*. *Proceedings of the National Academy of Sciences*, **114**(17):4507-4512. doi: 10.1073/pnas.1620432114.