Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Jul 22; 10(A):1373-1377.
https://doi.org/10.3889/oamjms.2022.8686
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Microbiology*

Научна фондација СПИРОСКИ
Scientific Foundation SPIROSKI
Since 2002

OPEN ACCESS

Detection of Atypical Motile *Staphylococcus aureus* from Rain Floods

Yusra Agha[1]*, Ashwak Hazem Najim[1], Reem Ayad Talaat[2], Shababa Abdulatife Bahjat[1]

[1]Department of Biology, College of Science, University of Mosul, Mosul, Iraq; [2]College of Environmental Sciences and Technology, University of Mosul, Mosul, Iraq

Edited by: Slavica Hristomanova-Mitkovska
Citation: Agha Y, Najim AH, Talaat RA, Bahjat SA. Detection of Atypical Motile *Staphylococcus aureus* from Rain Floods. Open Access Maced J Med Sci. 2022 Jul 22; 10(A):1373-1377. https://doi.org/10.3889/oamjms.2022.8686
Keywords: Colony spreading; Staphylococci; Rainwater; Oil displacement activity
***Correspondence:** Yusra Agha, Department of Biology, College of Science, University of Mosul, Mosul, Iraq. E-mail: yossbio56@uomosul.edu.iq
Received: 06-Apr-2022
Revised: 26-Jun-2022
Accepted: 12-Jul-2022
Copyright: © 2022 Yusra Agha, Ashwak Hazem Najim, Reem Ayad Talaat, Shababa Abdulatife Bahjat
Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist
Open Access: This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

Abstract

BACKGROUND: Heavy rain floods are one of the primary risk factors for human health, and it can significantly regulate microbial communities and enhance the transfer of infections within the affected areas. Recently, the flood crisis is becoming one of the severe natural events in Mosul/Iraq. It may continue for months during which samples of accumulated rainwater were collected.

AIM: We aimed to investigate atypical motile Staphylococcus aureus from rain floods in Mosul, Iraq.

METHODS: Twelve Staphylococcus aureus were isolated using two selective media; Mannitol Salt agar and Vogel-Johnson media in addition to Blood agar.

RESULTS: An unusual colony spreading which resembles "Bacillus" colonies in 12 Staphylococcus aureus isolates was observed on Mannitol Salt agar and semisolid nutrient agar. Actively motile cocci in single and cluster arrangements that is not characteristic of brownian movement was shown in wet mount microscopic observation Furthermore, biosurfactant detection by oil spreading method (oil displacement activity) showed that all isolates demonstrated various degrees of surfactant production which has been reported to be responsible for stimulating "colony spreading" phenomenon in S. aureus. Motility can play a crucial role for survival of bacterial species by which they get nutrients, avoid toxins and predators, and genetic information exchange by mating.

CONCLUSION: The present study highlights for the first time motile opportunistic S. aureus obtained from harvested rainwater samples during high-rainfall periods. Utilization of untreated harvested rainwater could thus offers a significant health threat to consumers, especially children and immunocompromised individuals.

Introduction

Natural environmental disasters like floods may cause disorders in the ecosystem that may lead to health hazards to human and animals all around the world. Floods are the most common and widespread environmental disasters [1], [2]. During a flood, many types of bacteria are accumulated and disposed by heavy rainwaters. The kind and level of contamination found in flood water varies markedly from one location to another and over time. A great deal depends on the size, nature, and site of contaminant sources in addition to the volume and route of flood waters. Stagnant floodwater can significantly affect the environmental microbiome and the extent of opportunistic pathogens spread [3]. Outbreaks of infectious diseases are the major concern after flooding. Also accumulated stagnate rain water leads to increasing number of vector-borne diseases (malaria and leishmaniasis) [4].

Pathogens found in rainwater belong to many bacterial species including the opportunistic *Staphylococcus aureus* [5]. This Gram-positive cocci can cause a wide range of both chronic and acute diseases ranging from superficial skin infections to life-threatening diseases such as sepsis and endocarditis. The ability of *S. aureus* to cause these infections is mainly due to their production of secreted cell wall-associated virulence factors such as proteins necessary for colonization, invasion, biofilm formation, or spreading throughout the host [6].

Most natural habitats of bacteria include a variety of animate and inanimate surfaces such as living tissues, soil particles, water, and air… Therefore, bacteria have established numerous mechanisms to translocate across such habitats and ranging from appendage-mediated motility to colony spreading.

Spreading staphylococci was first isolated in 1953 by [7]. The research work described spreading colonies as looking like a "bacillus colony". In 2007, Kaito reported that *S. aureus* can rapidly expand on soft agar surfaces. Colony spreading is one of the six classifications of bacterial translocation described by Henrichsen [8]. Because the colony spreading of *S. aureus* is due to multiple layers of growing cells, similar to the spreading of *B. subtilis* [9]. It was concluded that colony spreading is similar to, but distinct from, sliding, as it is independent of flagella or pili. *Staphylococcus aureus* is well known to be non-motile bacteria, but here we present observations that show it

is involved in a behavior which may classify it as being actively motile under certain conditions. Furthermore, Pollitt *et al.,* 2017, demonstrated that colonies of *S. aureus* can passively expand across the surface of sof agar plates, aided by the production of Phenol Soluble Modulin (PSM) surfactants [10], [11].

Events like flooding can increase the risk of vector-borne, viral respiratory, gastrointestinal, and soft tissue diseases. Overcrowded survival shelters results to infections such as measles, meningitis, tuberculosis, and influenza [4].

The purpose of the present study was to detect one of the most prevalent pathogen; *Staphylococcus aureus* in flood waters. This bacterium is capable of infecting almost every tissue and organ, causing a wide range of acute and chronic diseases and to observe the type of motility expressed as a response and adaptation to adverse environmental changes.

Materials and Methods

Sample collection

Samples were collected from puddles of accumulated rainwater at different locations in Mosul province following a period of heavy rains. The rainwater samples were obtained from the field site within 24 h after each rain event. Sterile containers were used to obtain the samples and were immediately transferred to the laboratory. Samples were streaked on Blood agar, and two selective media; Mannitol salt agar and Vogel-Johnson agar. Colonies with yellow colonies on Mannitol salt agar after 24 h incubation at 37°C which also gave black colonies on Vogel-Johnson agar and were coagulase positive were implemented for further studies.

Colony spreading assay

The spreading assay was performed as described by Kaito *et al.*, 2008, with some modifications [10], [12]. Nutrient broth supplemented with 0.24% agar was prepared and autoclaved. Sterile medium (25 ml) was poured onto a sterile petri dish. Plates were dried in a safety hood for 20 min before inoculation with bacteria. Overnight cultures of *S. aureus* were spot inoculated in the center of the plates and left to dry for 15 min and incubated at 37°C for 24–48 h. Colony spreading patterns were photographed using a 48 megapixel digital camera**.**

Detection of biosurfactant production by oil spreading assay

The oil spreading assay was developed by Morikawa *et al*., 2000. For this assay, 10 µl of crude oil is added to the surface of 20 ml of distilled water in a petri dish to form a thin oil layer. Then, 10 µl of culture was placed on the center of the oil layer. If the bacteria produce biosurfactant in the broth, the oil should be displaced and a clear zone is formed. The diameter of this clear zone on the oil surface is relative to biosurfactant activity. If biosurfactant is present in the cell free culture broth, the oil will be displaced with oil. The negative control was distilled water (without surfactant), in which no oil displacement or clear zone was observed. Oil displacement was captured by photos using digital camera [1].

Results and Discussion

Bacterial isolates

Twelve *S. aureus* bacterial isolates were isolated on all media used. The characteristic golden yellow colonies of mannitol fermenting *S. aureus* were shown to have a dendritic form of growth resembling that of Bacillus spp. colonies Figure 1. On performing wet mount on all isolates, it was shown that they were actively motile coccoid cells in single and cluster forms.

At the same time, colonies appeared in black colored colony on Vogel-Johnson agar; a selective medium used for identifying coagulase positive and mannitol-fermenting isolates (Figure 2).

Figure 1: Colony morphology (Dendritic spreading of colonies) of S. aureus on Mannitol salt agar following 48 h incubation at 37°C

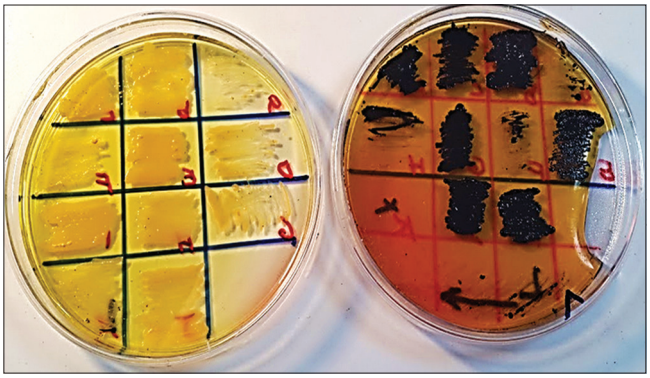

Figure 2: Colony morphology of S. aureus isolates on both Mannitol Salt agar and Vogel-Johnson agar

Colony spreading assay

Following spot inoculation of the previous isolates on semi-solid agar and a 24 h incubation period, diverse forms of dendritic spreading colonies were observed on each and every isolate and photographed. Some representative images are presented in Figure 3.

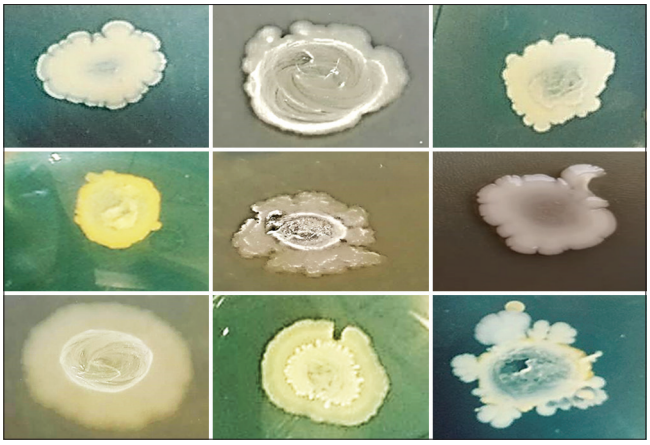

Figure 3: Colony spreading in S. aureus isolates on Nutrient broth +0.24% agar plates

S. aureus was considered to be non-motile because it lacks motility appendages (flagella and pilli,…). However, the present study demonstrates that *S. aureus* forms large spreading colonies on soft agar media; a phenomenon termed "colony spreading." This is in agreement with [10]. Although Kaito and Sekimizu found that *S. aureus* spreading only on TSA+ 0.24% agar plates, we found that *S. aureus* also spreads on Nutrient broth + 0.24% agar) as well.

This phenomenon was reported to be *controled* by the "*agr* locus," which is responsible for the expression of many exotoxins and adhesion proteins [11], [13], [14].

Bacterial motility is essential because it often indicates an adaptation necessary for survival and dissemination. It has been associated to colonization of many types of surfaces and hosts, antibiotic resistance correlated group behaviors and is also known to be involved in the virulence for a number of pathogenic bacterial species [15], [16]. Furthermore, colony spreading activity of *S. aureus* is important in understanding its translocation in human tissues to cause infections [17], [18].

Microscopic observation of *S. aureus* colonies in the present work clearly showed the structures described by Pollitt *et al*., 2015 [19] as "comets " which he defined as structures made of a number of cells that move across a surface seeding cells behind Figure 4. These structures are considered as the head start for dendrite formation. The tips of the comet are made of bacterial cells embedded in a slime matrix.

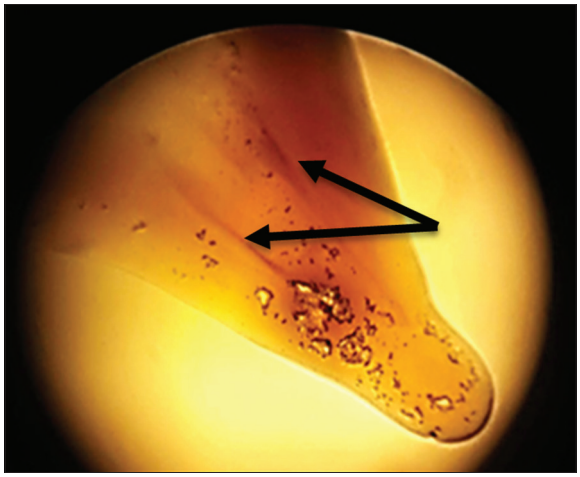

Figure 4: Comet structures showing S. aureus cells embedded in slime layer. Comets may etch the agar leaving a track behind them (arrow), and then grow into observable dendrites. Magnified 400× under compound light microscope

Pollitt *et al.,* 2015, demonstrated for the 1st time that *S. aureus* can form structures called "comets" on the surface of semisolid agar media. These structures are formed prior to dendrite formation and colony spreading. The tip of the comets is made of cells surrounded by a slime matrix and can etch the agar in some occasions as they move forward Figure 5.

The whole process occurs with no observable motility mechanisms (flagella or pilli). These results confirm that *S. aureus* can be actively motile [19]. Lin *et al.,* 2016, [20] proposed a mechanism for *S. aureus* spreading activity which involves extraction of water from the semisolid agar plate followed by quorum sensing as colony cells density increase. This leads to triggering transcription and formation of Phenolic Soluable Modulins (PSMs) which are cytolytic toxins that have surfactant properties [11], [18], [21].

In *S. aureus* eight PSMs have been identified which are controlled by the Agr system for quorum sensing [22]. Furthermore, a previous study by Kaito and Sekimizu [10] suggested that teichoic acids (lipoteichoic and wall teichoic acids) on the cell wall of *S. aureus* also play a role in the spreading motility of this bacterium. They might function as surfactants and reduce the friction between cells and the agar surface with the aid of water. Surfactants are known to weaken the water surface tension of the colony allowing water

to flow. Bacteria are then carried with the flowing water and spreads across the agar media. Rapid motility in nature is one of the virulence factors [15] that also aids the bacteria to obtain nutrients.

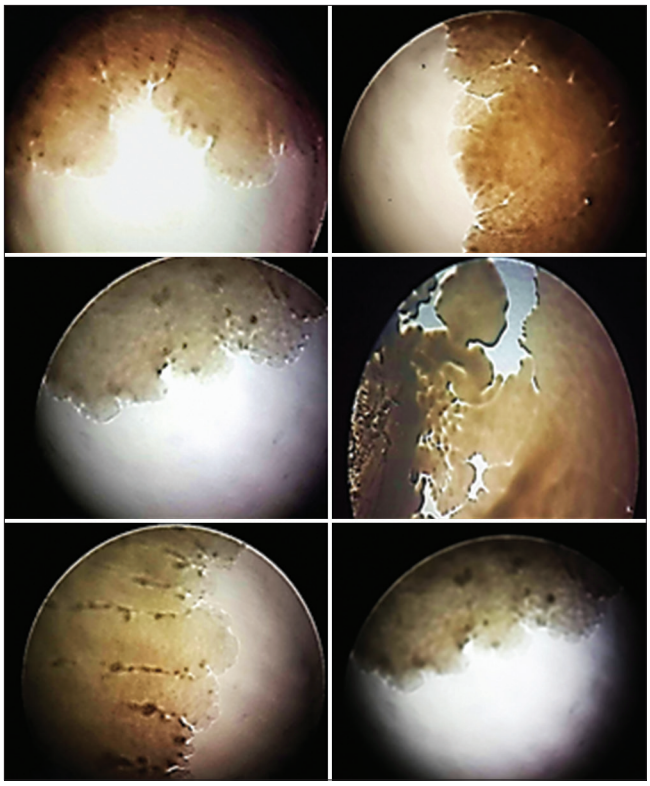

Figure 5: Dendrite formation of S. aureus in motility assay showing radial colony spread across semisolid agar. Magnification 400× under compound light microscope

As for *S. aureus* invasive strains, it is reasonable to predict that they could adopt their spreading motility to move away from implanted devices and colonize wet tissues of the human body [18].

Detection of biosurfactant production by oil spreading assay

This technique measures the diameter of clear zones caused when a drop of a biosurfactant-containing solution is placed on an oil–water surface. As biosurfactants have the ability to displace oil, it will form an oil spreading circle on the oil film which was seen under visible light and measured after 30 s [23]. All *S. aureus* isolated in the present study demonstrated various degrees of oil displacement as shown in Figure 6.

The oil spreading method is a reliable, simple, rapid, and requires no specialized equipment except a small volume of sample. It is very appropriate when the quantity of biosurfactant or activity is low [24], [25].

Pathogenic bacterial species being actively motile is of significance as motility mechanisms of all types have previously been shown to play an important role in virulence and colonization [15], [26].

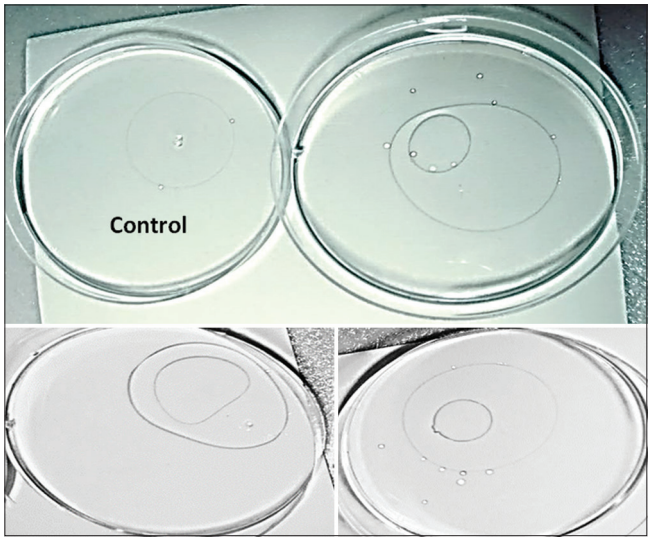

Figure 6: Oil displacement activity of S. aureus surfactant

Therefore, more research work is worth continuing as motility mechanisms are considered reasonable targets for vaccines and antimicrobial compounds. Finally, if *S. aureus* is actively motile, it would also be the first representative of Gram-positive bacteria with a characteristic Gram-positive cell wall which can move without flagella or pilli. Hence, other Gram-positive organisms may also be motile but yet undiscovered.

Conclusion

Staphylococcus aureus is historically diagnosed to be a nonflagellated, gram-positive pathogen which forms a biofilm on medical devices, such as catheters. The emergence of methicillin-resistant S. aureus, resistant to a wide range of antibiotics, is the source of serious health problems. In the present study, S. aureus was observed to demonstrate a type of spreading motility on the agar surface. Motility mechanisms of all types have previously been shown to play an important role in virulence and colonization. Motility could be a goal for new therapeutics and vaccines. Hence, provide new ways to attack S. aureus which is important because of the increased threatening antibiotic resistant strains. These findings also have significant impacts on understanding S. aureus behaviour in different habitats.

References

1. Okaka FO, Odhiambo BD. Relationship between flooding and out break of infectious diseases in Kenya: A review of the literature. J Environ Public Health. 2018;2018:5452938. https://doi.org/10.1155/2018/5452938
PMid:30416526

2. Paterson DL, Wright H, Harris PN. Health risks of flood disasters. Clin Infect Dis. 2018;67(9):1450-4. https://doi.org/10.1093/cid/ciy227

   PMid:30986298

3. Rodgers K, McLellan I, Peshkur T, Williams R, Tonner R, Hursthouse AS, *et al*. Can the legacy of industrial pollution influence antimicrobial resistance in estuarine sediments. Environ Chem Lett. 2019;17:595-607.

4. Shokri A, Sabzevari S, Hashemi SA. Impacts of flood on health of Iranian population: Infectious diseases with an emphasis on parasitic infections. Parasite Epidemiol Control 2020;9:e00144. https://doi.org/10.1016/j.parepi.2020.e00144

   PMid:32215322

5. Hamilton K, Reyneke B, Waso M, Clements Y, Ndlovu T, Khan W, *et al*. A global review of the microbiological quality and potential health risks associated with roof-harvested rainwater tanks. NPJ Clean Water. 2019;2:7. https://doi.org/10.1038/s41545-019-0030-5

6. Tsompanidou E, Denham EL, Sibbald MJ, Yang XM, Seinen J, Friedrich AW, *et al*. The Sortase A Substrates FnbpA, FnbpB, ClfA and ClfB antagonize colony spreading of *Staphylococcus aureus*. PLoS One. 2012;7(9):e44646. https://doi.org/10.1371/journal.pone.0044646

   PMid:22970276

7. Kruse E. Varianter af *Staphylococcus aureus* og albus. Nord Veterinaermed. 1953;5:982-94.

8. Henrichsen J. Bacterial surface translocation: A survey and a classification. Bacteriol Rev. 1972;36(4):478-503. https://doi.org/10.1128/br.36.4.478-503.1972

   PMid:4631369

9. Kinsinger RF, Shirk MC, Fall R. Rapid surface motility in *Bacillus subtilis* is dependent on extracellular surfactin and potassium ion. J Bacteriol. 2003;185(18):5627-31. https://doi.org/10.1128/JB.185.18.5627-5631.2003

   PMid:12949115

10. Kaito C, Sekimizu K. Colony spreading in *Staphylococcus aureus*. J Bacteriol. 2007;189(6):2553-7. https://doi.org/10.1128/JB.01635-06

    PMid:17194792

11. Tsompanidou E, Sibbald MJ, Chlebowicz MA, Dreisbach A, Back JW, Van Dijl JM, *et al*. Requirement of the *agr* locus for colony spreading of *Staphylococcus aureus*. J Bacteriol. 2011;193(5):1267-72. https://doi.org/10.1128/JB.01276-10

    PMid:21169484

12. Kaito C, Omae Y, Matsumoto Y, Nagata M, Yamaguchi H, Aoto T, *et al*. A novel gene, fudoh, in the SCCmec region suppresses the colony spreading ability and virulence of *Staphylococcus aureus*. PLoS One. 2008;3(12):e3921. https://doi.org/10.1371/journal.pone.0003921

    PMid:19079549

13. Morikawa M, Hirata Y, Imanaka T. A study on the structure-function relationship of lipopeptide biosurfactants. Biochim Biophys Acta. 2000;1488(3):211-8. https://doi.org/10.1016/s1388-1981(00)00124-4

    PMid:11082531

14. Ueda T, Kaito C, Omae Y, Sekimizu K. Sugar-responsive gene expression and the agr system are required for colony spreading in *Staphylococcus aureus*. Microb Pathog. 2011;51(3):178-85. https://doi.org/10.1016/j.micpath.2011.04.003

    PMid:21514374

15. Josenhans C, Suerbaum S. The role of motility as a virulence factor in bacteria. Int J Med Microbiol. 2002;291(8):605-14. https://doi.org/10.1078/1438-4221-00173

    PMid:12008914

16. Jarrell KF, McBride MJ. The surprisingly diverse ways that prokaryotes move. Nat Rev Microbiol. 2008;6(6):466-76. https://doi.org/10.1038/nrmicro1900

    PMid:18461074

17. Omae Y, Sekimizu K, Kaito C. Identification of *Staphylococcus aureus* colony-spreading stimulatory factors from mammalian serum. PLoS One. 2014;9(5):e97670. https://doi.org/10.1371/journal.pone.0097670

    PMid:24831518

18. Tsompanidou E, Denham EL, Becher D, De Jong A, Buist G, Van Oosten M, *et al*. Distinct roles of phenol-soluble modulins in spreading of *Staphylococcus aureus* on wet surfaces. Appl Environ Microbiol. 2013;79(3):886-95. https://doi.org/10.1128/AEM.03157-12

    PMid:23183971

19. Pollitt EJ, Crusz1 SA, Diggle SP. *Staphylococcus aureus* forms spreading dendrites that have characteristics of active motility. Sci Rep. 2015;5:17698. https://doi.org/10.1038/srep17698

    PMid:26680153

20. Lin MH, Ke WJ, Liu CC, Yang MW. Modulation of *Staphylococcus aureus* spreading by water. Sci Rep. 2016;6:25233. https://doi.org/10.1038/srep25233

    PMid:27125382

21. Otto M. Phenol-soluble modulins. Int J Med Microbiol. 2014;304(2):164-9. https://doi.org/10.1016/j. ijmm.2013.11.019

22. Chatterjee SS, Chen L, Joo HS, Cheung GY, Kreiswirth BN, Otto M. Distribution and regulation of the mobile genetic element-encodedphenol-soluble modulin PSM-mecin methicillin-resistant *Staphylococcus aureus*. PLoS One6. 2011;6(12):e28781. https://doi.org/10.1371/journal.pone.0028781

    PMid:22174895

23. Plaza G, Zjawiony I, Banat I. Use of different methods for detection of thermophilic biosurfactant-producing bacteria from hydrocarbon-contaminated bioremediated soils. J Petro Sci Eng. 2006;50(1):71-7. https://doi.org/10.1016/j.petrol.2005.10.005

24. Youssef NH, Duncan KE, Nagle DP, Savage KN, Knapp RM, McInerney MJ. Comparison of methods to detect biosurfactant production by diverse microorganisms. J Microbiol Methods. 2004;56(3):339-47. https://doi.org/10.1016/j.mimet.2003.11.001

    PMid:14967225

25. Laanto E, Bamford JK, Laakso J, Sundberg LR. Phage-driven loss of virulence in a fish pathogenic bacterium. PLoS One. 2012;7(12):e53157. https://doi.org/10.1371/journal.pone.0053157

    PMid:23308090

26. Margarit I, Rinaudo CD, Galeotti CL, Maione D, Ghezzo C, Buttazzoni E, *et al*. Preventing bacterial infections with pilus-based vaccines: The group B *Streptococcus* paradigm. J Infect Dis. 2009;199(1):108-15. https://doi.org/10.1086/595564

    PMid:19086816