

The Effect of Antibiotics and Shampoo on Different Bacterial Species Isolated From Healthy and Diseased Scalp of Humans

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Abstract

One hundred and ten bacterial isolates were obtained from 66 healthy and 44 diseased scalps from Al-Basrah peoples. *16SrRNA* gene sequences identified 79 isolates of 9 species of *B. cereus*, *S. aureus*, *K. pneumonia*, *S. pasture*, *S. epidermidis*, *E. faecalis*, *A. bivalvium*, *P. agglomerans* and *E. cloacae* were shared in both sources, 11 species of *P. pennerri*, *P. septica*, *S. sonnei*, *Flavobacterium sp.*, *E. hermanni*, *E. faecium*, *B. tropicus*, *B. subtilis*, *B. stutzeri*, *A. radioresistens* and *A. kooki* were only in diseased scalp comparison with 12 species of *B. pumilus*, *S. warneri*, *B. altitudinis*, *S. marcescens*, *B. enrichment*, *C. pulveris*, *E. hermanni*, *E. kobei*, *P. mirabilis*, *A. baumannii*, *P. ananatis* and *P. gavainiae* were only in healthy scalps. In general, *B. cereus* and *S. aureus* were the most frequent in both sources. However, 9 new global strains were published in National Center for Biotechnology Information and GenBank. The phylogenetic tree showed differences in the *16SrRNA* nucleotide sequence between each species isolated from the healthy and diseased scalp. Chemical shampoo appeared to be more effective (1017 colonies) to inhibit the bacteria isolates *In vitro* than medicated shampoo (1737 colonies). Cefotaxime, Vancomycin, Ciprofloxacin, Tetracycline and Imipenem were the best antibiotics to inhibit the bacterial species. Moreover, Erythromycin, Methicillin and Citizen were more effects toward healthy than diseased scalp isolates. The antibiotics typing for *S. aureus* isolates and RAPD test showed no identical strains spreading between both sources except the closely related strains No. 55- *S. aureus* from a diseased scalp and 83- *S. aureus* from a healthy scalp.

Keywords: antibiotic, shampoo, scalp, *16SrRNA*, RAPD.

1. Introduction

The human scalp contains diverse bacteria to influence both healthy and diseased scalps [1]. *Staphylococcus* and *Propionibacterium* were common scalp commensal microorganisms that are widely known as the cause of scalp disease in humans including common dandruff and seborrheic dermatitis [2]. There were few comprehensive studies on scalp bacteria and the complexity of normal commensal communities of the human scalp [3]. The abscess is a collection of pus that has formed within the body's tissue including scalp [4]. *Malassezia* and *Staphylococcus* were immediately inhibited by contact with 1% shampoo, Medicated shampoo is evidently effective against fungi as well as bacteria at all dilution levels revealing that it possessed very good lathering and cleaning ability. [5]. Bacteria isolated from scalp were successfully treated and rapidly cured with the combined topical multiple generations of

antibiotics (first, second, third and fourth generation) of piperacillin, tazobactam, cephalosporins, ciprofloxacin, levofloxacin, vancomycin, metronidazole and clindamycin [6]. However, it is important to differentiate between the strains of bacterial species especially when only a specific bacterial strain causes illness or resists to a specific antibiotic [7,8].

As a result to a large number of recent scalp infections and the difficulty to find appropriate treatment, this study tries to detect the associated bacteria with scalp diseases and their frequency. Furthermore, the suitable antibiotics for bacterial inhibition and the affected shampoo if possible.

2. Materials and Methods

2.1 Sample collection

One hundred and ten isolates were collected as 56 from a human healthy scalps and 54 from diseased scalps of clinics outpatients of the government hospitals in Basrah from 10 / 11 / 2020 to 15 / 5 / 2021. All samples were collected by sterile cotton swabs from the scalps and then placed in a falcon tube (Fisher scientific, USA) containing 4 ml of Brain heart infusion broth (Lab, UK) prepared to be a transporting medium, then sent to the laboratory to perform other tests.

2.2 Bacterial isolation and identification

Each sample was streaked on Brain heart infusion agar and Blood agar (lab, UK) plates, after 37 °C for 24 h. of incubation, cultured on Nutrient agar (Lab, UK) plates at 37 °C for 24 h. The grown colonies were stained with Gram's stain, subsequent for experiments and slants as stocks.

2.3 DNA extraction

After bacterial activated in Brain heart infusion broth and incubated at 37 °C for 24 h. the extraction was according to Presto™ Mini gDNA bacteria Kit (Geneaid, Taiwan). The distinguishing of DNA was through the agarose gel electrophoresis prepared by dissolving 0.25 gm of agarose powder in 25 ml of 1× TBE Buffer (1 part of 10 ×TBE to 9 parts of distilled water), heated for boiling on a hot plate until to be a clear mixture, 0.2 µl of ethidium bromide was added. Electrophoresed at 60 V for 30 min.

2.4 Amplification of 16S rDNA and sequencing

16S rDNA of the bacterial isolate was amplified according to [9]. Universal 16S rDNA primers (Alpha DNA, USA) were: 27 Forward 5'- AGAGTTTGATCCTGGCTCAG -3' and 1492 Reverse 5'-GGTTACCTTGTTACGACTT-3'. The mixture of PCR reaction contained 25 µl of Go Taq green master mix (Promega, USA), 3 µl (30 ng) of bacterial DNA, 2 µl (20 pmol) of each primer and the volume of the mix was adjusted to 50 µl with sterile water. Amplification was carried out by a Verity thermal cycler (Applied Biosystem, USA) with a program of initial denaturation at 95 °C for 5 min, 35 cycles (denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72 °C for 1 min) and final extension at 72 °C for 5 min. The amplified PCR bands were detected by electrophoresis at 1500 bp through 1% agarose gel at 60 V for 1 h and visualized under UV light.

Twenty µl of 16 S rDNA - PCR product of each sample was sent to the MacroGen company of South Korea “ <http://dna.microgen.com>” for sequencing. Bacteria at the species level were identified by BLAST “<https://blast.ncbi.nlm.nih.gov>” [10] after proofreading.

2.5 Phylogenetic tree

Twenty-six 16S rRNA sequences of 8 different bacterial species (only shared in the healthy and diseased scalp) identified in the present study were compared with 16SrRNA sequences of their reference strains from the GenBank by “CLUSTAL Omega” program <https://www.ebi.ac.uk/Tools/msa/clustalo/> [11]. The phylogenetic tree was accomplished by the MAFFT (Multiple Alignment using Fast Fourier Transform) program [12].

2.6 Effect of shampoo on the bacterial scalp

Thirty-two different bacterial species were tested for their sensitivity to the 100 % natural medicated shampoo (Herba Sense) and chemical shampoo (Head and shoulders) by procedure modified from [13]. For each shampoo, one colony of each bacterial species was cultured in 2 ml of Brain heart infusion broth at 37 °C for 24 h. A series of dilution (10^{-1} , 10^{-3} , and 10^{-6}) was prepared in sterilized Normal saline (0.9 %). 100 µl of each diluted suspension was transferred to the wall of the microtiter plate of 96 wells (Himedia, India) containing 100 µl of the shampoo. Nevertheless, one well was filled with 200 µl of bacterial suspension of BHIB without shampoo (control positive) and another well was filled with 100 µl of shampoo with 100 µl of Normal saline without bacteria (blank negative). The microtiter plate was incubated at 37 °C for 30 min. 10 µl from each well was spread on the Nutrient agar plate and incubated at 37°C for 24 h. The colonies were calculated.

2.7 Antibiotics sensitivity

Eleven antibiotics disc including Ciprofloxacin (CIP) 5 µg, Tetracycline (TE) 30 µg, Vancomycin (VA) 30 µg, Imipenem (IPM) 10 µg, Clindamycin (DA) 30 µg, Ampicillin (AM) 30 µg, Citizen (CZ) 30 µg, Cefotaxime (CTX) 30 µg, Erythromycin (E) 25 µg and Methicillin (METH) 30 µg (all of Mast diagnostics, UK) were prepared for 96 bacterial isolates using the method of disc diffusion on Muller Hinton agar (Lab, UK) as the information of clinical and laboratory standard institute [14].

2.8 Random amplified polymorphic DNA (RAPD)

Only seven *S. aureus* (No: 27, 83, 41,42, 43, 55 and 66) having the same congruent results of antibiotics sensitivity were undergo to RAPD technique using three primers (Alpha DNA, USA): OLP13 (5-ACCGCCTGCT-3), OLP6 (5-GAGGGAAGAG-3) and OLP11 (5-CGATGAGCC-3) to find the identical *S.aureus* strains by the procedure of [15]. The PCR reagent mixture of 25µl containing 1 µl of each primer, 3 µl of DNA template, 2 µl of Go Taq Green master mix (Promega, USA) and 7 µl of Nuclease Free water. The thermocycler (Applied Biosystem, USA) was accomplished with the steps for amplifying by starting with a cycle at 94°C for 5 min. 40 cycles of 93 °C for 1 min. 37° C for 90 sec. and 72°C for 1 min. Last extension was at 72°C for 7 min. The visible bands were shown by the UV transilluminator during agarose gel electrophoresis. The RAPD phylogenetic tree was accomplished using UPGMA

“www.http://genomes.urv.cat /UPGMA / ” to detect the distance between every two bands within the 7 strains [16].

3. Results and Discussion

3.1 Bacterial Identification

One hundred and ten isolates were collected from healthy 66 (60%) and disease 44 (40%), Gr+ ve was 38 (34.5%) and Gr -ve 16 (14.5%) in healthy, while 29 (26.4%) and 13 (11.8%) in disease, respectively. However, Gr + ve and Gr -ve in healthy were higher than in diseased scalp. Therefore, the total isolates in healthy 54 (49%) were higher than diseased scalp 42 (38.2%). all comparisons with significant differences in $P \leq 0.05$ (Table 1). The samples were cultured

Table 1- Frequency of Gram-positive and Gram-negative isolates in Healthy and diseased scalp

Bacterial Isolates	Healthy scalp		Diseased scalp	
	Gr +ve n(%)	Gr- ve n(%)	Gr +ve n(%)	Gr – ve n(%)
110	38 (34.5)	16 (14.5)	29 (26.4)	13 (11.8)
Total n(%)	54 (49)		42 (38.2)	

$P \leq 0.05$

on Brain heart infusion and Blood agar media to stimulate and support most bacterial growth [17, 18]. Scalp samples appeared Gr+ve to be higher than Gr-ve bacteria, this is agreed with several studies [19,20].

3.2 16S rRNA gene amplification and sequencing

The 16S rRNA gene bands of bacteria were shown in Figure (1). 16S rRNA was used to identify bacterial isolates because biochemical tests take a long time and may give false negative results, while 16SrRNA is a global strand for identifying species, classifying and showing the evolutionary relationships [21,22,23]. The results of only 79 successful 16S rRNA genes nucleotides sequencing of 32 different species with their frequency were presented in Table (2). The confidence similarity of identification with the type strains was $\geq 99\%$, while the unidentified bacteria were 31 isolates. In general, healthy and diseased scalps showed many

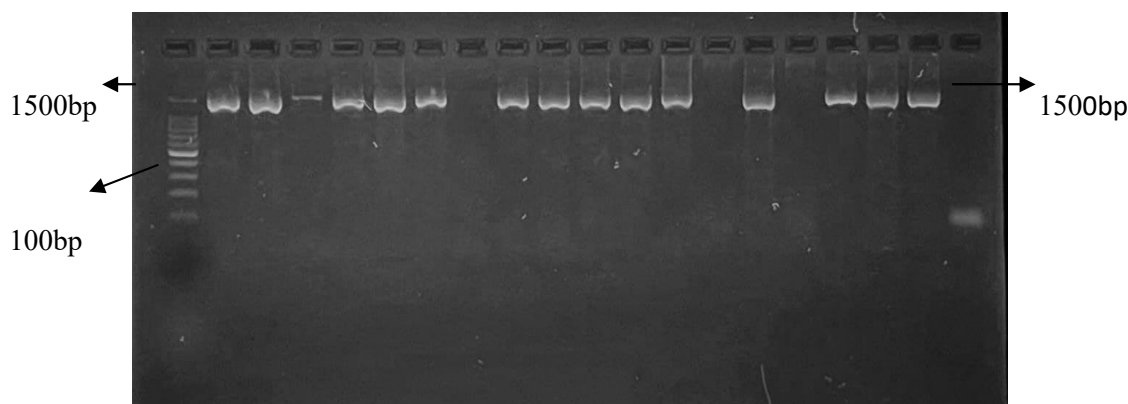


Figure 1- Agarose gel electrophoresis patterns shows PCR amplified products in a model of 16S rDNA (1500bp). Lane 1: 100 bp DNA ladder, other lanes with bands: 16S rDNA of bacterial isolates .

different bacterial species (n=32) because the water used for body washing containing different transported bacteria [24]. However, some of these bacterial species were found in healthy person and the different other in disease cases, emphasizing that the environment of the diseased scalp having some induced factors to grow those types of bacteria giving a good chance to be opportunistic at any time [25, 26]. However, most of the isolates were *Bacillus cereus* and *Staphylococcus aureus* were commensally on the scalp skin and can colonize to be opportunistic. Moreover, the variety of frequency in the different species due to their tolerance against salinity of tab water increasing the chance of bacterial to invite any injury in the scalp which may cause

Table 2- Frequency of bacterial isolates in diseased and healthy scalp

No	Bacteria species	n (%)	diseased scalp n (%)	healthy scalp n (%)
1	<i>Bacillus cereus</i>	16(14.5)	9(56.25)	7(43.75)
2	<i>Staphylococcus aureus</i>	9(8.1)	6(66.6)	3(33.4)
3	<i>Klebsiella pneumoniae</i>	6(5.4)	2(33.3)	4(66.7)
4	<i>Staphylococcus pasteurii</i>	5(4.5)	3(60)	2(40)
5	<i>Staphylococcus epidermidis</i>	5(4.5)	1(20)	4(80)
6	<i>Enterococcus faecalis</i>	3(2.7)	2(66.6)	1(33.4)
7	<i>Aeromonas bivalvium</i>	3(2.7)	2(66.6)	1(33.4)
8	<i>Pantoea agglomerans</i>	3(2.7)	1(33.4)	2(66.6)
9	<i>Enterobacter cloacae</i>	2(1.8)	1(50)	1(50)
10	<i>Proteus penneri</i>	1(0.9)	1(100)	0(0)
11	<i>Pantoea septica</i>	1(0.9)	1(100)	0(0)
12	<i>Shigella sonnei</i>	1(0.9)	1(100)	0(0)
13	<i>Flavobacterium sp</i>	1(0.9)	1(100)	0(0)
14	<i>Escherichia hermannii</i>	1(0.9)	1(100)	0(0)
15	<i>Enterococcus faecium</i>	1(0.9)	1(100)	0(0)
16	<i>Bacillus tropicus</i>	1(0.9)	1(100)	0(0)
17	<i>Bacillus subtilis</i>	1(0.9)	1(100)	0(0)
18	<i>Pseudomonas stutzeri</i>	1(0.9)	1(100)	0(0)
19	<i>Acinetobacter radioresistens</i>	1(0.9)	1(100)	0(0)
20	<i>Acinetobacter kookii</i>	1(0.9)	1(100)	0(0)
21	<i>Bacillus pumilus</i>	4(3.6)	0(0)	4(100)
21	<i>Staphylococcus warneri</i>	2(1.8)	0(0)	2(100)
23	<i>Bacillus altitudinis</i>	1(0.9)	0(0)	1(100)

24	<i>Serratia marcescens</i>	1(0.9)	0(0)	1(100)
25	<i>Bacterium enrichment</i>	1(0.9)	0(0)	1(100)
26	<i>Cronobacter pulveris</i>	1(0.9)	0(0)	1(100)
27	<i>Enterobacter hormaechei</i>	1(0.9)	0(0)	1(100)
28	<i>Enterobacter kobei</i>	1(0.9)	0(0)	1(100)
29	<i>Proteus mirabilis</i>	1(0.9)	0(0)	1(100)
30	<i>Acinetobacter baumannii</i>	1(0.9)	0(0)	1(100)
31	<i>Pantoea ananatis</i>	1(0.9)	0(0)	1(100)
32	<i>Pantoea gaviniae</i>	1(0.9)	0(0)	1(100)
33	*Gr+ve cocci	25(22.7)	1(2.3)	24(36.4)
34	*Gr+ve rode	1(0.9)	0(0)	1(1.5)
35	*Gr-ve rode	5(4.5)	5(11.4)	0(0)
Total n(%)		110	44(40)	66(60)

P≤0.05, *: Unidentified

bacteremia then blood poisoning [27]. On the other hand, 9 bacterial isolates were identified as new global strains published in NCBI, ENA and GenBank including isolate No.44- *B.cereus* strain IRQBAS111, 46- *B.cereus* strain IRQBAS110, 48-*B.cereus* strain IRQBAS109, 49- *S. sonnei* strain IRQBAS108, 60-*P.agglomerans* strain IRQBAS107, 61-*P.septica* strain IRQBAS106, 70-*S.epidermidis* strain IRQBAS105, 91-*P.agglolomerans* strain IRQBAS104, 92- *K. pneumonia* strain IRQBAS103.

3.3 Phylogenetic tree of bacterial species

The *16S rRNA* gene sequencing for 26 of 8 different bacterial species shared in both healthy and diseased scalps showed a close relatedness with each other and their type strain whether isolated from healthy or diseased scalp. But, interestingly, each species showed some differences in the 16S rRNA sequences for isolates from healthy than diseased scalps (Figure 2). In spite of each 8 (different species), the sharing both healthy and diseased sources, were in the same cluster but there were some differences in *16S rRNA* nucleotide sequences of species inside the same cluster, this could be boosted the hypothesis that the outer environment has the ability to effect on the bacterial genotype [28, 29].

3.4 Effect of shampoos on bacterial growth

Figure (3) showed a model of the effect of different types of shampoo on the bacteria colonies plates as a total sensitive “D” (No growth), intermediate sensitive “B” (some colonies), with the growth without shampoo “A” (control) and only shampoo without added bacteria “C” (blank) as in Table (3). In spite of the Head and Shoulders and Herba sense having the ability to inhibit bacteria compared to a primary culture (1017, 2737 and 6139 colonies, respectively) but, Head and shoulders was more effect than Herba sense, because the chemical shampoo contains many chemical materials could play a role to inhibit the bacteria. Therefore, it can be used for

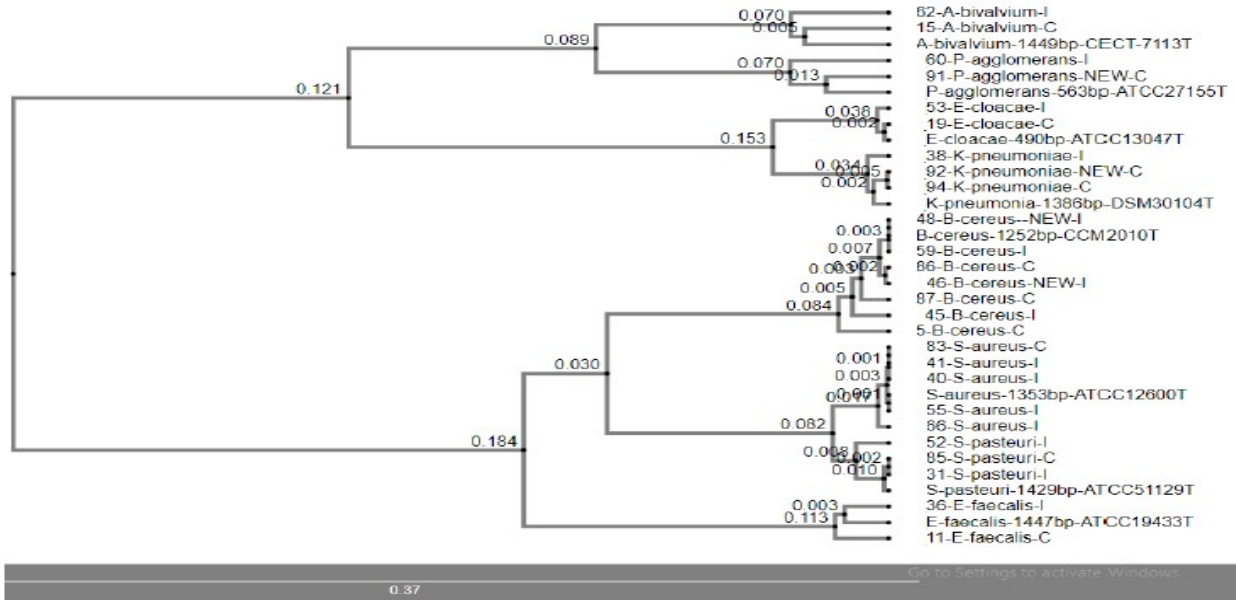


Figure 2- Rooted Neighbour-joining phylogenetic tree constructed sequences derived from an alignment of *16S rRNA* sequences of 26 of 8 different bacterial species (with different concatenation for each species) each including healthy and diseased scalp isolates with their type strain (T). The produced from a MAFT alignment was visualized using the forester version. The tree showed differences between the sequences of healthy and diseased scalps of each species even though bacterial species was in a separate cluster.

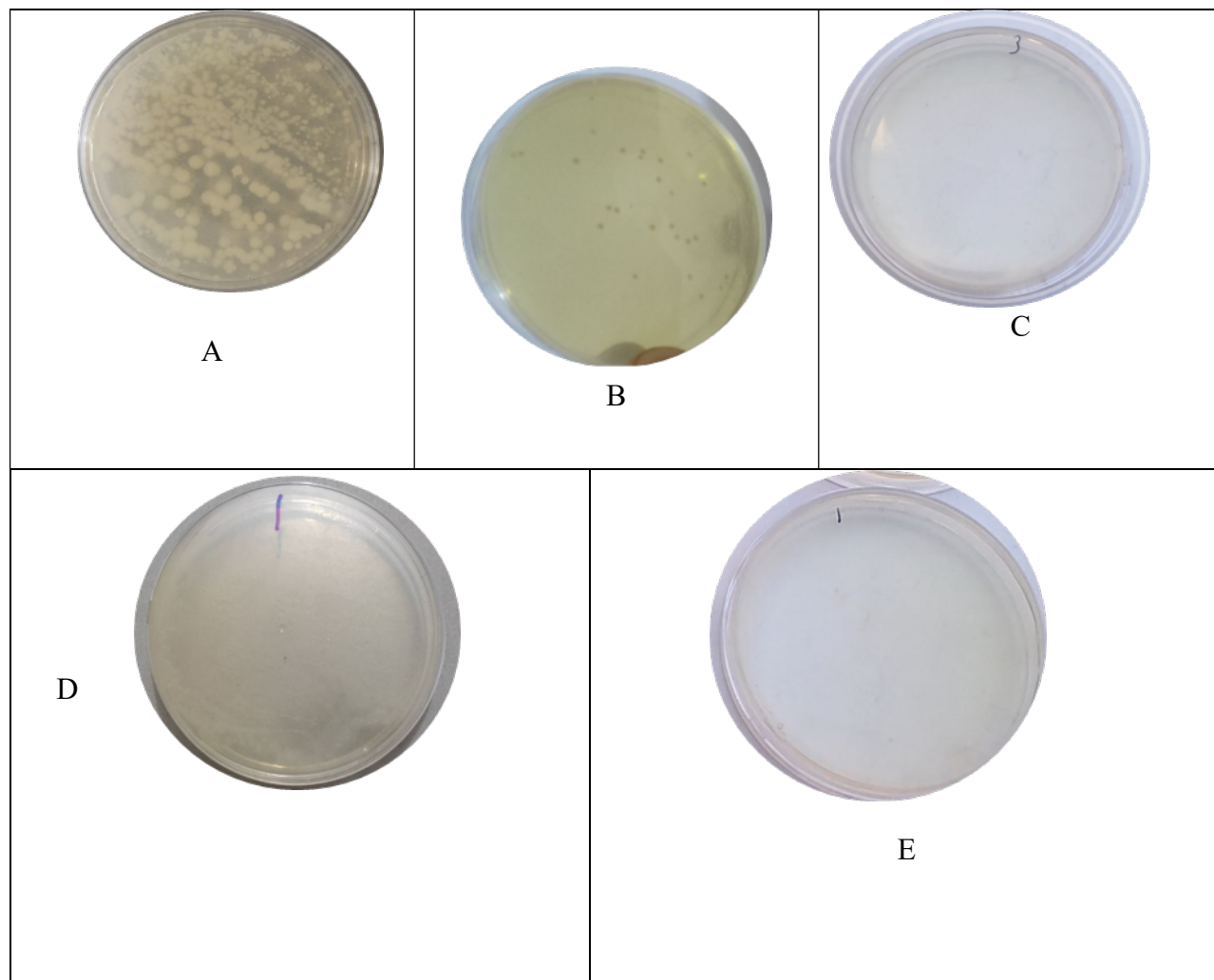


Figure 3- Model of the bacterial isolates treated with Herba sense shampoo showing total sensitive (D) and with Head and shoulders shampoo showing total sensitivity (E) or intermediate sensitivity (B). While, (A) as a control (primary culture) without shampoo and (C) as a blank of shampoo without added bacteria.

Table 3- Effect of medical shampoo (Herba sense) and chemical shampoo (Head and shoulders) on the bacterial species of scalp

No	no.of isolate	Primary culture (control) Before shampoo no.of colonies	With Herba sense Shampoo no.of colonies n(%)	With Head and shoulders shampoo no.of colonies n(%)	(Blank) no.of colonies n(%)
1	2- <i>B.pumilus</i>	194	56(28.8)	34(17.5)*	0(0)
2	3- <i>E.hermannii</i>	150	60(40)	48(32)*	0(0)

3	4- <i>C.pulveris</i>	190	39(20.5)	24(12.6)*	0(0)
4	9- <i>E.hormaechei</i>	85	0(0)	0(0)	0(0)
5	19- <i>E.cloacae</i>	340	45(13.5)	26(7.6)*	0(0)
6	20- <i>E.kobei</i>	169	60(35.5)*	82(48.5)	0(0)
7	21- <i>P.mirabilis</i>	80	0(0)	0(0)	0(0)
8	24- <i>A.baumannii</i>	245	44(17.9)*	49(20)	0(0)
9	25- <i>P.gaviniae</i>	335	95(28.3)	26(7.7)*	0(0)
10	28- <i>P.penneri</i>	263	65(43.7)	68(25.8)*	0(0)
11	32- <i>Flavobacterium</i> <i>sp</i>	102	20(19.6)	0(0)*	0(0)
12	33- <i>E.faecium</i>	220	98(44.5)	45(20.4)*	0(0)
13	36- <i>E.faecalis</i>	186	85(45.6)	42(22.5)*	0(0)
14	39- <i>P.stutzeri</i>	188	92(48.9)	65(34.5)*	0(0)
15	42- <i>B.subtilis</i>	210	89(42.3)	48(22.8)*	0(0)
16	48- <i>B.cereus</i>	260	65(20)*	92(35.3)	0(0)
17	49- <i>S.sonnei</i>	94	9(9.5)	0(0)*	0(0)
18	52- <i>S.pasteuri</i>	195	62(31.7)	26(13.3)*	0(0)
19	54- <i>B.tropicus</i>	290	85(29.3)	38(13.1)*	0(0)
20	55- <i>S.aureus</i>	192	46(23.3)	28(14.5)*	0(0)
21	57- <i>B.altitudinis</i>	95	20(21)	0(0)*	0(0)
22	61- <i>P.septica</i>	110	26(23.6)	0(0)*	0(0)
23	62- <i>A.bivalvium</i>	95	0(0)	0(0)	0(0)
24	65- <i>A.kookii</i>	240	96(40)	45(18.7)*	0(0)
25	69- <i>A.radioresistens</i>	150	53(35.3)	0(0)*	0(0)
26	71- <i>S.warneri</i>	190	65(34.2)	0(0)*	0(0)
27	80- <i>B.enrichment</i>	255	55(21.5)	45(17.6)*	0(0)
28	89- <i>S.epidermidis</i>	245	76(31.0)	65(26.5)*	0(0)
29	91- <i>P.agglomerans</i>	205	94(45.8)	60(29.2)*	0(0)
30	92- <i>K.pneumoniae</i>	265	92(34.7)	35(13.2)*	0(0)
31	95- <i>S.marcescens</i>	105	0(0)*	26(24.7)	0(0)
32	96- <i>Pantoea</i> <i>ananatis</i>	196	45(22.9)	0(0)*	0(0)
	Total	6139	1737(28.3)	1017(16.6)*	0(0)

P ≤ 0.05

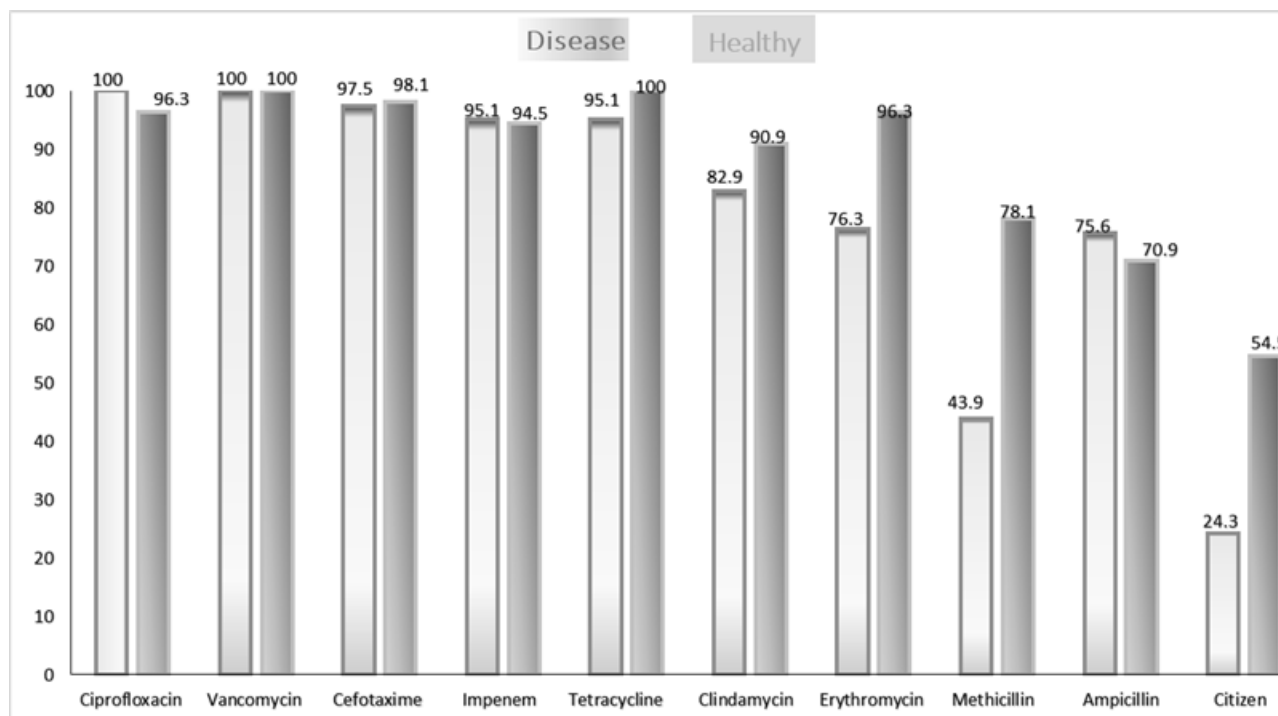
preventing and treating scalp disorders and diseases such as dandruff, on the other hand, the medicated shampoo had pronounced conditioning effects on hair as well as a good cleaning and lathering ability [30].

3.5 Antibiotic sensitivity

There were no significant differences in the positive effect of the antibiotics Ciprofloxacin, Vancomycin, Cefotaxime, Imipenem, Tetracycline, Clindamycin and Ampicillin toward bacterial isolates from diseased (n=43) and healthy scalps (n=53) respectively, in agreement with [31,32]. On the other hand, the isolates from healthy scalps were more sensitive to the antibiotics Erythromycin, Methicillin and Citizen than those isolated from diseased scalps respectively, [33,34] as Figure (4).

3.6 Detection the identical *S. aureus* strains by antibiotic sensitivity and Random Amplified Polymorphic DNA (RAPD-PCR)

The antibiotics susceptibility for all (9) *S. aureus* species showed identical results between isolates No.27 and 83, 55 and 66, and among 40, 41 and 43 (Table 4). RAPD technique (Figure 5) was performed with 7 *Staphylococcus aureus* isolates that have identical patterns of antibiotic susceptibility. The phylogenetic tree of the two strains No.55 and 83 from diseased and healthy scalps respectively, were closely related, but the other strains of the two sources were considered as not related (Figure 6). The distance of the matrix of the bands of RAPD-PCR for strains 55 and 83 of *S. aureus* was 0.614 as listed in Table (5). As procedure steps of [35], the present study elected *S. aureus* to determine the congruence among the strains isolated from the scalp. Since, *S. aureus* has a high pathogenicity among other bacteria. Primarily, the antibiotic susceptibility for the 9 *S. aureus* isolates showed 3 different identical groups of 7 strains including No. 27 and



P<0.05

Figure 4- Comparison between the antibiotics sensitivity toward bacterial isolates from diseased and healthy scalps

Table 4- Antibiotic sensitivity and resistance of bacterial isolated (*S. aureus*)

No	no of isolates	DA	METH	E	CTX	CIP	IPM	VA	AM	CZ	TE
1	*10	R	R	S	S	S	S	S	S	R	S
2	*27	S	S	S	S	S	S	S	S	S	S
3	**40	R	R	S	S	S	S	S	R	R	S
4	**41	R	R	S	S	S	S	S	R	R	S
5	**43	R	R	S	S	S	S	S	R	R	S
6	**55	S	R	S	S	S	S	S	S	R	S
7	**66	S	R	R	S	S	S	S	R	R	S
8	**67	S	R	R	S	S	S	S	R	R	S
9	*83	S	S	S	S	S	S	S	S	S	S

* (10, 27 and 83): *S.aureus* from healthy scalp, ** (40, 41, 43, 55, 66 and 67): *S.aureus* from diseased scalp

Ciprofloxacin (CIP) Vancomycin (VAN), Cefotaxime (CTX), Imipenem (IPM), Tetracycline (TE), Clindamycin (DA), Erythromycin (E), Methicillin (METH), Ampicillin (AM), Citizen (CZ).

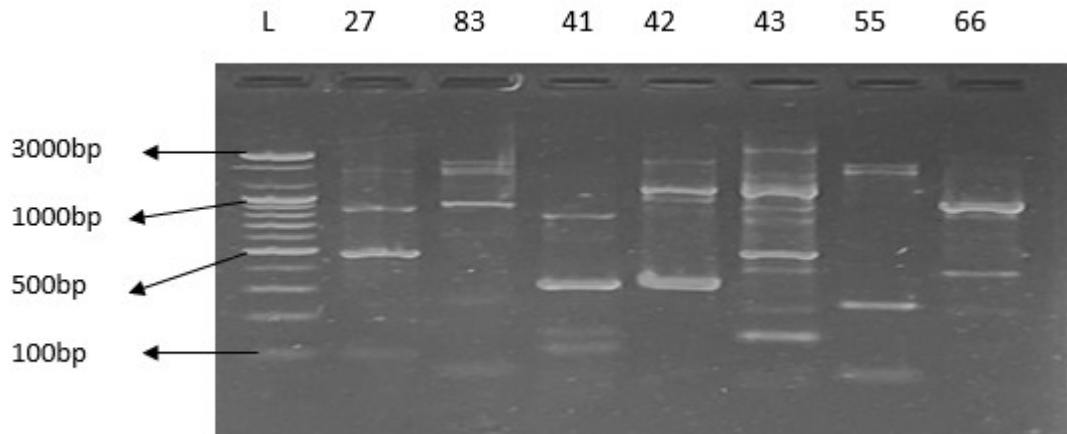


Figure 5- Agarose gel electrophoresis for RAPD patterns bands of *Staphylococcus aureus*. Lane L: 100 bp Marker, Lane 27 and 83, 41,42 and 43, 55 and 66: RAPD gene bands of *S. aureus* strains.

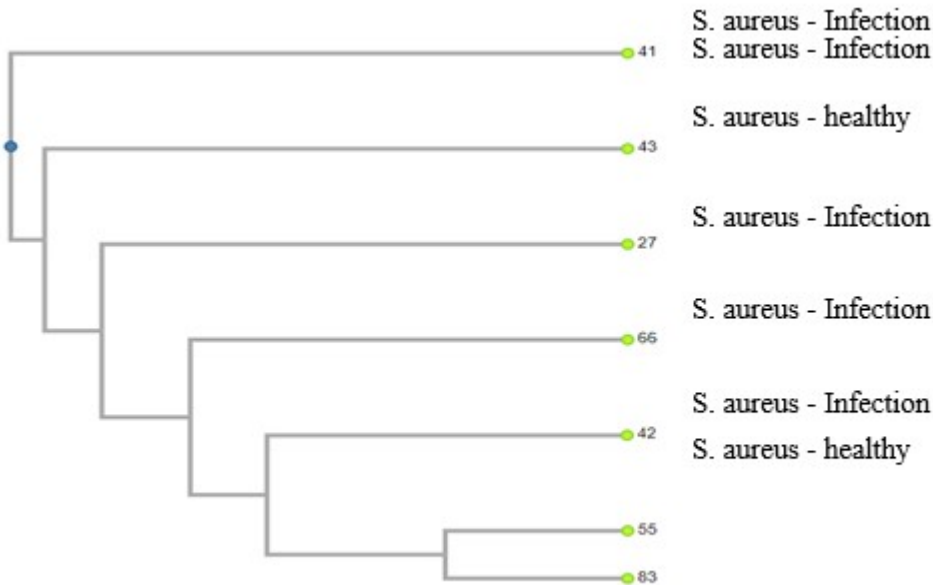


Figure 6- Dendrogram of 7 *S. aureus* strains 27, 41, 43, 66, 55, 83 and 42 constructed by a set of variables RAPD bands using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm. Bootstrap values after 100 repetitions. Strain 55 and 83 were closely related.

Table 5- Distance Matrix among RAPD bands of *S. aureus* strains

no. of isolate	27	83	41	42	43	55	66
27	0	1.457	1.977	1.816	2.071	1.677	2.130
83		0	1.928	1.473	2.049	0.614	1.674
41			0	1.688	2.368	2.261	2.239
42				0	1.942	0.953	1.486
43					0	1.803	1.945
55						0	1.253
66							0

41, 42, 43, 55, and 66 – *S. aureus* from diseased scalp, 27 and 83 – *S. aureus* from healthy scalp. 0,614 refer to the lowest variations among number and bands distances

83, 55 and 66, and 40, 41 and 43 while the other 2 strains No. 1 and 67 appeared too different from any strains. Secondly, the RAPD test of 7 strains showed a close relation between strain No. 55-*S.aureus* from infection and 83-*S.aureus* from a healthy scalp. In the exception of this relation, the results refer to the constantly changing in the bacterial genome of the scalp as a result of the environmental factors affect and more importantly, there was no signal that the isolates transport among human scalps. However, the relatedness of clinical and environmental strains is dependent on the genetic similarity between strains from both sources [36].

Conclusions

In spite of there being many bacterial species shared in both healthy and diseased scalps, but there are many other species found in the source not in others and vice versa. However, the type of shampoo and the suitable antibiotics are very useful to inhibit the bacterial scalp. The genomic mutations in the bacterial strains refer to the effect of the scalp environment on the genomic strains causing a difficulty to detect the genetic relations to find the ancestor.

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References

1. Park, T., Kim, H. J., Myeong, N. R., Lee, H. G., Kwack, I., Lee, J., Kim, B. J., Sul W. J. & An, S. (2017). Collapse of human scalp microbiome network in dandruff and seborrhoeic dermatitis. *Exp Dermatol*, 26 (9): 835-838.
2. Wang, L., Clavaud, C., Bar-Hen, A., Cui, M., Gao, J., Liu, Y., Liu, C., Shibagaki, N., Gueniche, A., Jourdain, R., Lan, K., Zhang, C., Altmeyer, R. & Breton, L., (2015).

- Characterization of the major bacterial-fungal populations colonizing dandruff scalps in Shanghai, China, shows microbial disequilibrium. *Exp. Dermatol*, 24: 398.
3. Perez Perez, G. I., Gao, Z., Jourdain, R., Ramirez, J., Gany, F., Clavaud, C., Demaude, J., Breton, L. M. & Blaser, J. (2016). Body Site Is a More Determinant Factor than Human Population Diversity in the Healthy Skin Microbiome, *PLoS*, 11: e0151990.
 4. Graili, P., Ieraci, L., Hosseinkhah, N. & Argent-Katwala, M. (2021). "Artificial Intelligence in Outcomes Research: A Systematic Scoping Review." *Expert Review of Pharmacoeconomics and Outcomes Research*, 21 (4): 601–623.
 5. Leong, C., Schmid, B., Buttafuoco, A., Glatz, M. & Bosshard, P. P. (2019). *In vitro* efficacy of antifungal agents alone and in shampoo formulation against dandruff-associated *Malassezia* spp. and *Staphylococcus* spp. *Int J Cosmet Sci*, 41(3): 221-227.
 6. Kyriakopoulos, A. M., Nagl, M., Orth-Höller, D., Marcinkiewicz, J., Baliou, S., & Zoumbourlis, V. (2020). Successful treatment of a unique chronic multi-bacterial scalp infection with N-chlorotaurine, N-bromotaurine and bromamine T. *Access Microbiology*, 2 (7): acmi000126.
 7. Abd Al-Abbas, M. J., & Chemagh, A. A. (2014). Molecular Genetic Study Confirming the Transmission of Nasopharyngeal Bacteria to Middle Ear in Patients with Chronic Supportive Otitis Media, Including New Global Strains in GenBank: MunaAla1, MunaAla2, IRQBAS5 and IRQBAS6. *International Journal of Pharmaceutical Research and Bioscience*, 3 (2): 379-97.
 8. Fida, M., Wolf, M. J., Hamdi, A., Vijayvargiya, P., Garrigos, Z. E., Khalil, S., Greenwood-Quaintance, K. E., Thoendel, M. J. & Patel, R. (2021). Detection of Pathogenic Bacteria From Septic Patients Using 16S Ribosomal RNA Gene-Targeted Metagenomic Sequencing. *Clin Infect Dis*, 73 (7):1165-1172.
 9. Miyoshi, T., Iwatsuki, T., & Naganuma, T. (2005). Phylogenetic characterization of 16S rRNA gene clones from deep-groundwater microorganisms that pass through 0.2-micrometer-pore-size filters. *Applied and environmental microbiology*, 71 (2): 1084-1088.
 10. Kerbauy, G., Perugini, M., Yamauchi, L. M. & Yamada-Ogatta, S. F. (2011). Vancomycin-dependent *Enterococcus faecium* *vanA*: characterization of the first case isolated in a university hospital in 141 Brazil. *Brazilian Journal of Medical Biological Research*, 44: 253- 257.
 11. Becker, K., Harmsen, D., Mellmann, A., Meier, C., Schumann, P., Peters, G., & Von Eiff, C. (2004). Development and evaluation of a quality-controlled ribosomal sequence database for 16S ribosomal DNA-based identification of *Staphylococcus* species. *Journal of clinical microbiology*, 42: 4988-4995.
 12. Katoh, K., Misawa, K., Kuma, K. I., & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic acids research*, 30 (14): 3059-3066.

13. Corona, A., Persico, P., Vercelli, A., Gramenzi, A., & Cornegliani, L. (2020). In vitro antimicrobial activity of a black currant oil based shampoo versus a chlorhexidine 4% shampoo on bacteria strains isolated from canine pyoderma: A comparative study. *Insights in Veterinary Science*, 4 (1): 014-017.
14. CLSI. (2017). Performance standards for antimicrobial susceptibility testing M100. Journal.
15. Zare, S., Derakhshandeh, A., Haghkhah, M., Naziri, Z. & Broujeni, A. M. (2019). Molecular typing of *Staphylococcus aureus* from different sources by RAPD-PCR analysis. *Heliyon*, 5 (8): e0223128.
16. Garcia-Vallvé, S. & Puigbo, P. (2009). Dendro UPGMA: a dendrogram construction utility. Universitat Rovirai Virgili. 1-14.
17. Hamad, M. A., Hussein, S. A., Mahmmoud, E. N., & Al-AAlim, A. M. (2020). The inhibitory role of effective microorganisms on the growth of pathogenic bacteria. *Iraqi Journal of Veterinary Sciences*, 34 (1): 153-158.
18. Khalfallah, G., Gartzten, R., Möller, M., Heine, E., & Lütticken, R. (2021). A new approach to harness probiotics against common bacterial skin pathogens: Towards living antimicrobials. *Probiotics and Antimicrobial Proteins*, 13 (6): 1557-1571.
19. Ozkan, J., Nielsen, S., Diez-Vives, C., Coroneo, M., Thomas, T., & Willcox, M. (2017). Temporal stability and composition of the ocular surface microbiome. *Scientific reports*, 7 (1): 1-11.
20. Teweldemedhin, M., Gebreyesus, H., Atsbaha, A. H., Asgedom, S. W., & Saravanan, M. (2017). Bacterial profile of ocular infections: a systematic review. *BMC ophthalmology*, 17 (1): 1-9.
21. Boudewijns, M., Bakkers, J. M., Sturm, P. D., & Melchers, W. J. (2006). 16S rRNA gene sequencing and the routine clinical microbiology laboratory: a perfect marriage ?. *Journal of clinical microbiology*, 44 (9): 3469-3470.
22. Bosshard, P. P., Zbinden, R., Abels, S., Boddingtonhaus, B., Altwegg, M., & Bottger, E. C. (2006). 16S rRNA gene sequencing versus the API 20 NE system and the VITEK 2 ID-GNB card for identification of nonfermenting Gram-negative bacteria in the clinical laboratory. *Journal of clinical microbiology*, 44 (4): 1359-1366.
23. Wu, Y. W. (2018). ezTree: an automated pipeline for identifying phylogenetic marker genes and inferring evolutionary relationships among uncultivated prokaryotic draft genomes. *BMC genomics*, 19 (1): 7-16.
24. Abd Al Wahid, Z., & Abd Al-Abbas, M. J. (2019). Detection of E. Coli Strains Isolated from Water Sources and Diarrhea Cases by Random Amplified Polymorphic DNA in Basrah Governorate. *J International Journal of Sciences*, 8: 68-83.
25. Rajput, M., & Kumar, N. (2020). In vitro Antimicrobial and antibiofilm efficacy of medicinal plant extracts against clinical MDR isolates from scalp infection cases. *Int J Sci Technol Res*, 9 (2): 4218-4228.

26. Kyriakopoulos, A. M., Nagl, M., Orth-Höller, D., Marcinkiewicz, J., Baliou, S., & Zoumbourlis, V. (2020). Successful treatment of a unique chronic multi-bacterial scalp infection with N-chlorotaurine, N-bromotaurine and bromamine T. *Access Microbiology*, 2 (7): acmi000126.
27. Agyare, C., Boamah, V. E., Zumbi, C. N., & Osei, F. B. (2018). Antibiotic use in poultry production and its effects on bacterial resistance. *Antimicrobial resistance—A global threat*, 33-51.
28. Marzorati, M., Wittebolle, L., Boon, N., Daffonchio, D., & Verstraete, W. (2008). How to get more out of molecular fingerprints: practical tools for microbial ecology. *Environmental microbiology*, 10 (6): 1571-1581.
29. Bodor, A., Bounedjoum, N., Vincze, G. E., ErdeinéKis, Á. Laczi, K., Bende, G., ... & Rákhely, G. (2020). Challenges of unculturable bacteria: environmental perspectives. *Reviews in Environmental Science and Bio/Technology*, 19 (1): 1-22.
30. Igwebike-Ossi, C. D., Iroha, I. R., & Oke, B. (2017). Formulation and antimicrobial activity of triclosan-based medicated shampoo. *Journal of Chemical and Pharmaceutical Research*, 9 (7): 100-104.
31. El Shallaly, G. H., Hassan, A. N., Siddig, N. O., Mohammed, R. A., Osman, E. A., & Ibrahim, M. A. (2012). Study of patients with community-acquired abscesses. *Surgical Infections*, 13 (4): 250-256.
32. Fadare, F. T., Elsheikh, E. A., & Okoh, A. I. (2022). In Vitro Assessment of the Combination of Antibiotics against Some Integron-Harboring Enterobacteriaceae from Environmental Sources. *Antibiotics*, 11 (8): 1090.
33. Khalid, M., Junejo, S., & Mir, F. (2018). Invasive Community Acquired Methicillin-Resistant *Staphylococcal aureus* (CA-MRSA) Infections in Children. *J Coll Physicians Surg Pak*, 28 (9): 174-177.
34. Li, F., Wu, Y., Bian, W., Huang, L., Zhu, X., Chen, X., & Wang, M. (2020). Features and associated factors of bacterial skin infections in hospitalized patients with pemphigus: a single-center retrospective study. *Annals of Clinical Microbiology and Antimicrobials*, 19 (1): 1-9.
35. Mahdi, M. A., Abd Al-Abbas, M. J., & Alsamak, A. M. (2021). Distribution of *OatA* alleles detected by a new designed primer in bacteria isolated from eye infections in Basrah governorate/Iraq. *Annals of the Romanian Society for Cell Biology*, 25 (3): 8258-8277.
36. Lee, C. N., Chen, W., Hsu, C. K., Weng, T. T., Lee, J. Y. Y., & Yang, C. C. (2018). Dissecting folliculitis (dissecting cellulitis) of the scalp: a 66-patient case series and proposal of classification. *JDDG: Journal der Deutschen Dermatologischen Gesellschaft*, 16 (10): 1219-1226.