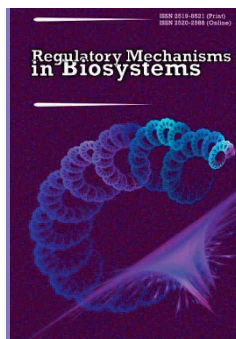

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2024, 15(3), 548–551
doi: 10.15421/022476

Biotyping and genotyping of seven strains of *Cutibacterium acnes*

S. A. Mahmood, G. A. Mohammad

University of Mosul, Mosul, Iraq

Article info

Received 30.05.2024
Received in revised form 04.07.2024
Accepted 25.07.2024

Department of Biology, College of Science, University of Mosul, Mosul, 41001, Iraq.
Tel.: +964-770-822-84-64.
E-mail: Salim.23scp107@student.uomosul.edu.iq, Kadsbio32@uomosul.edu.iq

Mahmood, S. A., & Mohammad, G. A. (2024). Biotyping and genotyping of seven strains of Cutibacterium acnes. Regulatory Mechanisms in Biosystems, 15(3), 548–551. doi:10.15421/022476

Bacterial typing is the process of distinguishing between different strains, it is essential for epidemiology, identifying source of infection and guiding treatment. This study aimed to detect the biotypes and genotypes for seven strains of *Cutibacterium acnes* obtained from the University of Mosul, already isolated from individuals with acne vulgaris in Mosul city (Iraq) and diagnosed by the molecular method based on 16S rRNA gene. Accuracy in biotyping depended on which type of the sugar (ribose, erythritol and sorbitol) was consumed by *C. acnes*, each sugar was placed separately in culture media with 1% concentration in the presence of phenol red to detect sugar fermentation. The *C. acnes* isolates were cultured anaerobically for 3 days at 37 ºC. The results showed that four biotypes (I, III, IV and V) emerged in our isolates indicated by the change in color of the medium from orange to yellow depending on the fermentable sugar. The distribution percentage of bacteria among these biotype was dominated by the V biotype (42.8%), while the III biotype was percentage (28.6%) and the I and IV biotypes each took up a lesser percentage (14.3%). It is worth mentioning that the II biotype didn't appear at all in any of the isolates. For genotype, it was based on genes (16s rRNA, *ATPase*, *atpD*, *Toxin Fic family*, *recA*, and *soda*), DNA was extracted and special primers were used, then they were amplified by PCR technique, after electrophoresis, the resulting bands showed that the seven isolates of *C. acnes* were distributed into two genotypes only. One of them is I A2 which the isolate CS6 belonged to, another genotype is a new one- it is worth noting that it is prevalent in six isolates out of seven, and contains (16s rRNA, *ATPase*, *atpD*, *recA*, and *sodA*) genes, and because it is a new genotype that was unique to our isolates in this study, we called it IV genotype. The conclusion of this study is that the biotyping and genotyping of *C. acnes* differed from other typing in another countries, therefore, caution should be exercised in using the appropriate treatment depending on the prevailing condition in the country and not adhering to what is known theoretically regarding the causes of infections.

Keywords: biotype; genotype; IV genotype; acne vulgaris; multiplex polymerase chain reaction.

Introduction

Acne vulgaris is a chronic inflammation affecting the pilosebaceous unit, which often starts around adolescence, and is caused by the gram-positive bacteria named *Cutibacterium acnes*. Because of the potential central role of *C. acnes* in acne, emerging key elements related to its genomic and phenotypic heterogeneity open the way to deeply explore the role of its different phylotypes in acne development, and provide new insights on the cellular physiology underlying this pathogenesis (Scholz & Kilian, 2016).

To track bacterial outbreaks in healthcare facilities, germs must be identified and differentiated beyond species level, a process known as bacterial typing (Schürch et al., 2018). Typing is a method of microbial characterization used to distinguish between strains of a bacterial species, bacterial strain typing is a critical component of epidemiological surveillance and investigation, strain typing can confirm epidemiological links in disease outbreaks and provide insights into the dynamics of the bacterial population (de Filippis, 2022). There are many typing techniques that have been adopted such as: antibiotics sensitivity, biotyping, phage typing, bacteriocin typing, protein typing, serotyping, and multi locus enzyme electrophoresis (MLEE), genotyping and whole genome sequence etc. (Nutman & Marchaim, 2019; Sharma et al., 2020; Karlsen et al., 2023). The increasing cost of molecular techniques is one of the biggest barriers to their use, but at the same time, they are considered the most accurate of all the techniques (Aboulela & El-Soudany, 2023). Several studies have examined different molecular typing techniques including multi locus variable number-tandem repeat analysis, whole genome multi locus sequence typing and core genome single nucleotide polymorphism (Rumore et al., 2018; Gateau et al., 2019; van Alphen et al., 2021). Higaki (2003) divided the *C. acnes* into five biotypes: I, II, III, IV or V depending on the results of sugar fermentation test for sorbitol, ribose, and erythritol. Furthermore, *C. acnes* can be classified into a number of genotypes, designated types IA1, IA2, IB, IC, II and III, which appear to display differences in their association with specific types of infections (Barnard et al., 2015).

According to previous studies, fermentation tests can be utilized for biotyping (Higaki et al., 2004) while using multiplex polymerase chain reaction (PCR) for genotyping (Lomholt & Kilian, 2010). In view of the above mentioned importance of this topic and the benefits to be gained in determining the epidemiology and control of bacteria, and also due to the insufficient information regarding typing of *C. acnes*, especially locally in Mosul city, the aim of this research is to investigate the predominate biotype and genotype in seven strains of *C. acnes* isolated from acne vulgaris in Mosul city.

Materials and methods

Seven strains of *C. acnes* named (CS1, CS2, CS3, CS4, CS5, CS6 and CS7) studied previously molecularly by 16s rRNA gene were obtained from Department of Biology, College of Science, University of Mosul.

In order to find out which biotype of *C. acnes* is prevalent in skin infection in Mosul, Zhang's method was followed with some modifications, determining of the biotyping was achieved by preparing the basal medium (urea agar base) supplemented with Tween 80 (25 µL per 100 mL) and vitamin K (1 mg per 100 mL). One of the three sugars (ribose, erythritol and sorbitol) was added separately in concentration (1 g of carbohydrate per 100 mL). A color – fermenting regent (phenol red) was placed in the

medium with a concentration 0.025 g/L to indicate the fermentation of the sugar by bacteria. Finally, the pH was fixed to 7. *Cutibacterium acnes* strains were cultured and incubated anaerobically in jar with gas pack (Microxpress, Spain) to provide anaerobic condition at 37 ºC for 3 days (Zhang et al., 2019).

If the strain fermented all sugars, then it will belong to the first (I) biotype, If the strain ferments ribose and erythritol, it will belong to the second (II) biotype, if the strain ferments ribose and sorbitol only, it will belong to the third (III) biotype, if the strain ferments only ribose, it will belong to the fourth (IV) biotype, finally if the strain does not ferment any of three sugar, it will belong to the fifth (V) biotype.

In order to conduct genotyping of the our seven local strains of *C. acnes* to know their genetic origin, or at least the degree of relatedness of their epidemiology in our society, six genes were dependent: 16s rRNA *ATPase*, *atpD*, *Toxin*, *Fic family*, *recA*, and *sodA* gene were tested. These genes were detected using their primers provided by (Macrogene, Korea) as their sequences shown in Table 1 depended on the Barnard et al. (2015).

Table 1
The sequence of genotyping primers and their genes size

Primers name	Primers sequence (5'-3')	Genes size, bp
16s rRNA	F-AGAGTTTGATCMTGGCTCAG R-AAGGAGGTGATCCARCCGCA	1495
ATPase	F-GCGTTGACCAAGTCCGCCGA R-GCAAATTCGCACCGCGGAGC	494
atpD	F-TCCATCTGGCCGAATACCAGG R-TCTTAACGCCGATCCCTCCAT	351
Toxin, Fic family	F-AGGGCGAGGTCCTCTTCTACCAGCG R-ACCCTCCAACTGCAACTCTCCGCCT	305
recA	F-GCGCCCTCAAGTTCTACTCA R-CGGATTTGGTGATAATGCCA	225
sodA	F-CGGAACCATCAACAAACTCGAA R-GAAGAACTCGTCAATCGCAGCA	145

DNA was extracted using DNA extraction kit (Geneaid, Taiwan) following the steps recommended by the manufacturing company. The concentration and purity of DNA were measured using Nano drop device (Biodrop, England).

Thirty Microleter reaction mixtures were prepared, containing 2.0 µL (< 250 ng) of DNA, 15 µL of Green Master Mix (1X) (Promega, USA), all primers were placed in a PCR tube in volume 1 µL from each one as shown in the (Table 2).

Table 2
Reaction mixture of multiplex PCR with final concentration

Components	Volume in µL	Final concentration
DNA sample	2	<250 ng
16s r RNA primer	2 (1 Reverse + 1 Forward)	0.3 µM
ATPase primer	2 (1 Reverse + 1 Forward)	0.3 µM
atpD primer	2 (1 Reverse + 1 Forward)	0.3 µM
Toxin, Fic family primer	2 (1 Reverse + 1 Forward)	0.3 µM
recA primer	2 (1 Reverse + 1 Forward)	0.3 µM
sodA primer	2 (1 Reverse + 1 Forward)	0.3 µM
Master mix (2X)	15	1X
Nuclease free water	1	–
Total volume	30	–

The amplification program was carried out following (Barnard et al., 2015), then optimization for annealing temperature were changed from 66 to 60, to 58, lastly 55 ºC was the degree needed to obtain the typical result. Subsequently and multiplex PCR program for 16s rRNA *ATPase*, *atpD*, *Toxin*, *Fic family*, *recA*, and *sodA* genes was set as follows: initial denaturation at 94 °C for 1 min followed by 35 cycles of amplification including a denaturation step at 94 °C for 45 s, annealing at 55 °C for 45 s and extension at 72 °C for 1 min. A final extension step was set at 72 °C for 3 min. The amplification was done by thermos cycler (Humburg, Germany).

Agarose gel electrophoresis of PCR products was accomplished for 16s rRNA (1495bp) ATPase (494 bp), atpD (351 bp), recA (225bp) and sodA (145 bp) genes on the 2% of agarose at 60 v in 60 minutes, after that the UV-Transilluminator (Muv 21-312, Taiwan) apparatus was used to view the bands.

Results

After anaerobic cultivation of *C. acnes* in the medium prepared containing single type of sugars with neutral acidity (pH = 7), our local strains of *C. acnes* had different biotypes distributed to four biotypes (I, III, IV and V) as revealed in Figure 1 these results relied on the changing of the medium's color from orange to yellow due to the sugar ferment type that was added in the medium. However, the biotype II was not revealed among our local strains.

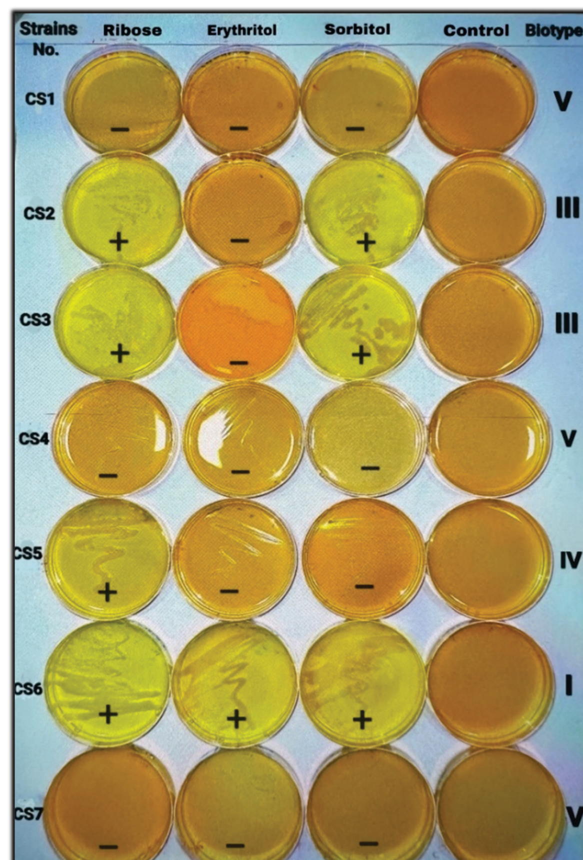

Fig. 1. Four biotype of local seven strains of *C. acnes*

Although the number of isolates is small, the percentage of biotypes was calculated as shown in Table 3 based on Figure 1 where three strains of *C. acnes* belong to fifth biotype, two strains belong to third biotype, one isolate for each first biotype and fourth biotype.

Table 3
Biotyping patterns of *C. acnes* strains

Biotype	I	II	III	IV	V
Strains of *C. acnes*	CS6	(not found)	CS2 CS3	CS5	CS1 CS4 CS7
Biotype percentage	14.3%	0 %	28.6%	14.3%	42.8%

After the investigation of genes approved for genotyping of the local strains of *C. acnes*, which are: 16s rRNA, *ATPase*, *atpD*, *Toxic Fic family*, *recA*, and *sodA*, only two genotypes were obtained, one of theme is I A2 genotype.

The electrophoresis revealed the presence of fluorescent bands belonging to all genes for six strains of *C. acnes* except the gene (*Toxic Fic family*) which didn't appear in any strain at all.

The *C. acnes* CS6 showed a difference from the rest of the strains in that it contained only three genes: 16s rRNA, ATPase and soda, depending on standard ladder. Figure 2 demonstrates the bands closed approximately to 145, 225, 351, 494, 1495 bps for six strains belong to *sodA*, *recA*, *atpD*, *ATPase*, 16s rRNA respectively, and only 145, 494, 1495 bps for the *C. acnes* CS6 belong to *sodA*, *ATPase*, 16s rRNA respectively.The band with molecular weight 305 bp belonging to (*Toxic fic family*) gene did not appear in all strains.

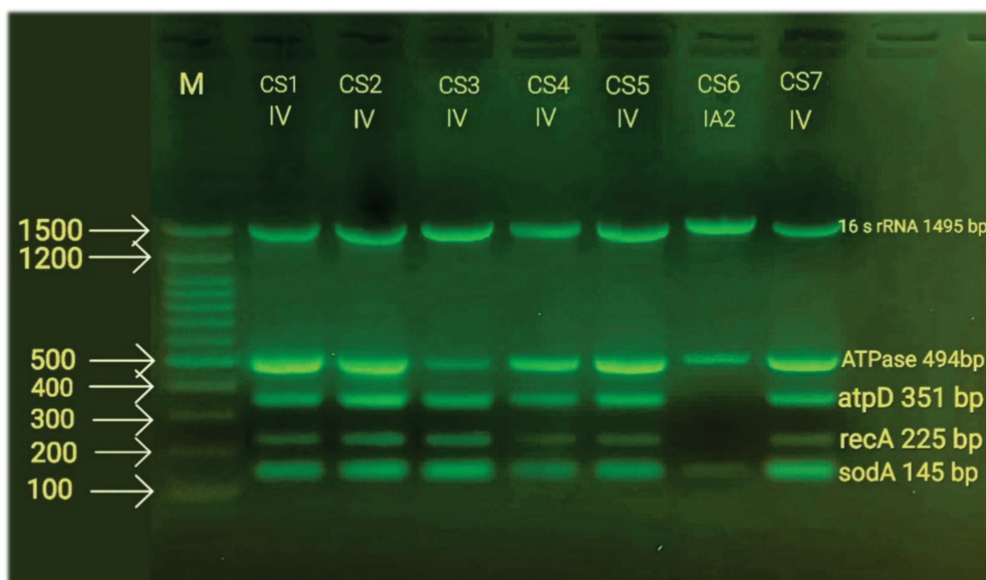

Fig. 2. Agarose gel electrophoresis of PCR products for 16s rRNA, *ATPase*, *atpD*, *recA* and *sodA* genes on the 2% of agarose at 60 v in 60 minutes: M – standard ladder, wells from CS1–CS7; our local strains of *C. acnes* while IA2 and IV represent genotype

Discussion

The typing is crucial in microbiology because of many reasons such as epidemiological detection, for treatment, for research and to control bacterial outbreak. Some researches have dealt with bacterial profiling of many bacterial species, not limited to *C. acnes* like (*Acinetobacter baumannii* and *Campylobacter jejuni*, etc. (Mohammad & Al-Rawe 2005; Ahmed & Mohammad, 2021).

As mentioned in materials and methods, *C. acnes* CS6 belonged to the first (I) biotype, due to its ability to ferment the three sugars (ribose, erythrotol and sorbitol) and changed the color of the medium to yellow in the three plates. In addition, two strains of *C. acnes* No. CS2 and CS3 demonstrated their ability to ferment ribose and sorbitol, which changed the color of the media from orange to yellow, that represents the third (III) biotype, while *C. acnes* CS5 changed the color of the medium containing ribose only to yellow due to its ability to ferment this sugar only and did not ferment others, therefore it belonged to the fourth (IV) biotype, lastly the *C. acnes* CS1, CS4 and CS7 belonged to the fifth (V) biotype because they could not ferment any of sugars: ribose, erythrotol and sorbitol.

Higak (2003) obtained four different biotypes from *C. acnes* isolated from Japan, the third (III) biotype was the most predominant with 50% in his study, while the first (I) and second (II) biotypes had a prevalence of 20%. However, the presence of the fourth (IV) biotype was only 10%. lastly, the fifth (V) biotype did not appear in his results in contrast to our study with local isolates that gave this pattern the most predominant one.

Zhang et al. (2019) indicated after that conducting biotyping of *C. acnes* isolated from Huashan Hospital (Shanghai, China), the results of the third (III) biotype was the most predominant with 50.8% followed by the first (I) and fifth (V) biotype with 15.9%, the second (II) biotype with 12.7% and lastly the fourth (IV) biotype with 4.8%.

Nagy et al. (2006) obtained only two biotypes of *C. acnes* that were isolated from (Szeged, Hungary) where most of the patterns were of the first (I) biotype with 75% and the second (II) biotype with 25%.This diversity is considered small compared to our study.

The variation in the predominance of bacterial biotypes may be attributed to the type of dermis from which this bacterium is isolated, where the skin could be oily or dry, depending on the amount of sebum secreted by individuals infected with acne. Additionally, it may be related to the extent to which the affected skin is exposed to weather conditions, which can influence the nature of the skin, depending on the geographical location of the country.

This experiment of genotyping was carried out based on Barnard et al. (2015), whose results revealled several genotypes distributed according to the presence of bands in different patterns in their strain to six genotypes, they called the first four of them, genotype (I) with subdivision: I A1 included (16s rRNA, *ATPase*) genes, I A2 included (16s rRNA, *ATPase*, *sodA*) genes, I B included (16s rRNA, *sodA*) genes, I C included (16s rRNA, *ATPase*, *Toxin Fic family*) genes, II second genotype included (16s rRNA, *atpD*) genes, Finally genotype III included (16s rRNA, *recA*) genes (Barnard et al., 2015).

In our study, due to the emergence of a new genotype which differs from those in the previously mentioned studies and which we consider to be reported for the first time, we suggest adding a sixth pattern namely of *C. acnes*, which means that the local strains had a single genetic origin that may not exist in another country.

Only one local isolate belonged to the genotype I A2 because it contains three genes (16s rRNA, *ATPase*, *sodA*) and this type is present in the studies mentioned later.

The more genes present in the strain, the greater the additional survival characteristics, because every gene present in the bacterium is encoded into an adaptive gene, resistant or virulence factor, and each of the four genes that were detected in this study carries a specific trait, as: ATPase – gene that is responsible for converting the chemical energies stored in ATP to mechanical actions within the cell (Rappas,2004). *sodA* – gene that encodes the enzyme superoxide dismutase constitutes a very important antioxidant defense against oxidative stress in the body. *sodA* – gene is responsible for protecting a particular bacterial strain from hydrogen peroxide-induced oxidative damage and promotes its pathogenicity (Saleh, 2023). *atpD* – gene, which encodes for ATP synthase, is among the most conserved genes (Algammal et al., 2021). *recA* which encodes for the *RecA* protein is a recombinase that helps bacteria repair their DNA through recombinational means. *recA* is controlled on several levels. However, the *Toxin Fic family* gene, which was not present in the local strains in this study, is encoded into a virulence factor that gives the bacteria greater pathogenicity, because it secretes *Fic* proteins as toxins that mediate post-translational modifications of host cell proteins (Veyron et al., 2018).

All genotypes were obtained in different percentage by Barnard et al. (2015), 312 strains of *C. acnes* isolated from Los Angeles (USA) were used to confirm the multiplex PCR, the I A1 was dominant with 46.4%, I A2 with 6.4%, IB with 20.2%, IC with 2.2%, genotype II with 14.4% and genotype III with 10.4%.

Zhang et al. (2019) conducted the same experiment to detect the genotyping of their strains. They isolated 63 *C. acnes* strains from Huashan Hospital in Shanghai (China).They obtained percentages that differed from Barnard et al. (2015), and all patterns were revealed in their results except for the genotype III, which was not present in their strains. IA1 was the predominant genotype with 71.4%, followed by IA2 with 19.0%, II genotype with 4.8%, IB with 3.2%, and IC with 1.6%. Oliveira et al. (2024) obtained only two genotypes from eight strains of *C. acnes* isolated

from Portugal, where the IA2 genotype was dominant with 75% and the other genotype was II with 25%.

Genotyping techniques are crucial for research in epidemiology, disease surveillance, infectious disease transmission and can identify genetic factors associated with transmission, antibiotic resistance in humans and animals and disease prevalence in populations. These insights help develop effective prevention and treatment strategies, molecular epidemiology is commonly utilized in infectious illnesses to identify the source of infection and the ancestral ties between individuals within a group, scientific applications of coalescent theory and phylogeographic analysis include pandemics and nosocomial outbreaks. Microorganism-isolated sequencing data is crucial for phylogenetic analysis and infectious disease studies. Phylodynamics is maturing. Molecular phylogenetic and evolutionary approaches are needed (Ciccozzi et al., 2019).

Conclusion

Bacterial typing is considered important because it helps in understanding the characteristics and metabolism of bacteria. It can assist in estimating potential health risks in addition of identifying the species and classifying them. Moreover, typing, especially genotyping, plays an important role in epidemiology.

Our strains had four different biotypes (I, II, III, IV and V) and the biotype II was not revealed in our study. For genotyping, two different genotypes were obtained, the first pattern belonged to genotype (IA2) and the second pattern was a new genotype that varies from those found in the previously stated researches. Therefore it is possible to suggest a new pattern to be added to the six discovered patterns and this was given the name IV genotype, which is represented by (16s rRNA, *ATPase*, *atpD*, *recA*, and *sodA*). By typing bacteria, we can track the spread of infectious diseases, understand the causes of transmission, and help take measures to control epidemics.

The authors thanks staff of the College of Science, University of Mosul (Iraq).

The authors declare they have no conflicts of interest.

References

Aboulela, A. G., & El-Soudany, I. (2023). Non-sequencing based molecular bacterial typing as an affordable tool for outbreak investigation in low-income countries. Microbes and Infectious Diseases, 4(1), 97–107.

Ahmed, N. H., & Mohammad, G. A. (2021). Detection of some virulance genes and variation of *Acinetobacter baumannii*. Middle East Journal of Science, 7(1), 64–79.

Algammal, A. M., Hashem, H. R., Alfifi, K. J., Hetta, H. F., Sheraba, N. S., Ramadan, H., & El-Tarabili, R. M. (2021). *atp* D Gene sequencing, multidrug resistance traits, virulence-determinants, and antimicrobial resistance genes of emerging XDR and MDR-*Proteus mirabilis*. Scientific Reports, 11(1), 9476.

Al-Rawe, A. M., & Al-Taee, G. A. (2005). Biotyping some *Campylobacter jejuni* isolates and their sensitivity to common antibiotics. Rafidain Journal of Science, 16(12), 31–38.

Barnard, E., Nagy, I., Hunyadkürti, J., Patrick, S., & McDowell, A. (2015). Multiplex touchdown PCR for rapid typing of the opportunistic pathogen *Propionibacterium acnes*. Journal of Clinical Microbiology, 53(4), 1149–1155.

Ciccozzi, M., Lai, A., Zehender, G., Borsetti, A., Cella, E., Ciotti, M., Sagnelli, E., Sagnelli, C., & Angeletti, S. (2019). The phylogenetic approach for viral infectious disease evolution and epidemiology: An updating review. Journal of Medical Virology, 91(10), 1707–1724.

de Filippis, I. (Ed.). (2022). Molecular typing in bacterial infections. Vol. 2. Springer International Publishing, Cham.

Gajdács, M., Spengler, G., & Urbán, E. (2017). Identification and antimicrobial susceptibility testing of anaerobic bacteria: Rubik's cube of clinical microbiology. Antibiotics, 6(4), 25.

Gateau, C., Deboscker, S., Couturier, J., Vogel, T., Schmitt, E., Muller, J., Ménard, C., Turcan, B., Zaidi, R. S., Youssouf, A., Lavigne, T., & Barbut, F. (2019). Local outbreak of *Clostridioides difficile* PCR-ribotype 018 investigated by multi locus variable number tandem repeat analysis, whole genome multi locus sequence typing and core genome single nucleotide polymorphism typing. Anaerobe, 60, 102087.

Higaki, S. (2003). Lipase inhibitors for the treatment of acne. Journal of Molecular Catalysis B: Enzymatic, 22(5–6), 377–384.

Higaki, S., Nakamura, M., Morohashi, M., & Yamagishi, T. (2004). *Propionibacterium acnes* biotypes and susceptibility to minocycline and Keigai-rengyo-to. International Journal of Dermatology, 43(2), 103–107.

Karlsen, S. T., Rau, M. H., Sánchez, B. J., Jensen, K., & Zeidan, A. A. (2023). From genotype to phenotype: Computational approaches for inferring microbial traits relevant to the food industry. FEMS Microbiology Reviews, 47(4), fuad030.

Li, W., Raoult, D., & Fournier, P. E. (2009). Bacterial strain typing in the genomic era. FEMS Microbiology Reviews, 33(5), 892–916.

Lomholt, H. B., & Kilian, M. (2010). Population genetic analysis of *Propionibacterium acnes* identifies a subpopulation and epidemic clones associated with acne. PloS One, 5(8), e12277.

Nagy, I., Pivarcsi, A., Kis, K., Koreck, A., Bodai, L., McDowell, A., Seltmann, H., Patrick, S., Zouboulis, C. C., & Kemény, L. (2006). *Propionibacterium acnes* and lipopolysaccharide induce the expression of antimicrobial peptides and proinflammatory cytokines/chemokines in human sebocytes. Microbes and Infection, 8(8), 2195–2205.

Nutman, A., & Marchaim, D. (2019). How to: Molecular investigation of a hospital outbreak. Clinical Microbiology and Infection, 25(6), 688–695.

Oliveira, A. S., Gaspar, C., Rolo, J., Palmeira-de-Oliveira, R., Teixeira, J., Martinez de Oliveira, J., & Palmeira-de-Oliveira, A. (2024). Comparative efficacy of essential oils against *Cutibacterium acnes*: Effect upon strains from phylotypes with different virulence patterns. Journal of Ethnopharmacology, 287, 114935.

Rappas, M., Niwa, H., & Zhang, X. (2004). Mechanisms of ATPases – a multidisciplinary approach. Current Protein and Peptide Science, 5(2), 89–105.

Rumore, J., Tschetter, L., Kearney, A., Kandar, R., McCormick, R., Walker, M., Peterson, C. L., Reimer, A., & Nadon, C. (2018). Evaluation of whole-genome sequencing for outbreak detection of verotoxigenic *Escherichia coli* O157:H7 from the Canadian perspective. BMC Genomics, 19, 870.

Saleh, T. F., & Altaey, O. Y. (2023). Histomorphometrical and histochemical study of caecum in adult Muscovy ducks (*Cairina moschata*). Advances in Animal and Veterinary Sciences, 11(6), 1021–1029.

Scholz, C. F., & Kilian, M. (2016). The natural history of cutaneous propionibacteria, and reclassification of selected species within the genus *Propionibacterium* to the proposed novel genera *Acidipropionibacterium* gen. nov., *Cutibacterium* gen. nov. and *Pseudopropionibacterium* gen. nov. International Journal of Systematic and Evolutionary Microbiology, 66(11), 4422–4432.

Schürch, A. C., Arredondo-Alonso, S., Willems, R. J. L., & Goering, R. V. (2018). Whole genome sequencing options for bacterial strain typing and epidemiologic analysis based on single nucleotide polymorphism versus gene-by-gene-based approaches. Clinical Microbiology and Infection, 24(4), 350–354.

Sharma, A., Lee, S., & Park, Y. S. (2020). Molecular typing tools for identifying and characterizing lactic acid bacteria: A review. Food Science and Biotechnology, 29, 1301–1318.

Van Alphen, L. B., von Wintersdorff, C. J., & Savelkoul, P. H. (2021). Epidemiological typing using WGS. In: Application and integration of omics-powered diagnostics in clinical and public health microbiology. Springer International Publishing, Cham. Pp. 69–87.

Veyron, S., Peyroche, G., & Cherfils, J. (2018). FIC proteins: From bacteria to humans and back again. Pathogens and Disease, 76(2), fty012.

Zhang, N., Yuan, R., Xin, K. Z., Lu, Z., & Ma, Y. (2019). Antimicrobial susceptibility, biotypes and phylotypes of clinical cutibacterium (formerly propionibacterium) acnes strains isolated from acne patients: An observational study. Dermatology and Therapy, 9, 735–746.