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Rīgas Stradiņa universitāte

Renāta Vīksne (Klagiša)

Biofilmu nozīme recidivējoša tonsilīta un paratonsilārā abscesa patoģenēzē un klīniskajā norisē

Promocijas darbs zinātnes doktora grāda "zinātnes doktors (*Ph. D.*)" iegūšanai

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Nozare – klīniskā medicīna
Apakšnozare – otorinolaringoloģija

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Anotācija

Vispārējā populācijā tonsilīts pēc rinofaringīta un vidusauss iekaisuma ir trešā biežākā infekcija, kuras dēļ pacienti vēršas pie otorinolaringologa. Tonsilīta epizodēm atkārtojoties vairākkārt, akūts tonsilīts pāriet recidivējošā tonsilītā (RT). RT paasinājumu ārstēšanā lieto antibiotikas. Pašlaik kā galvenais noteicošais faktors pacientu atlasē tonsilektomijai tiek izmantots paasinājumu biežums pēdējo trīs gadu laikā. Ja ir septiņas tonsilīta paasinājuma epizodes gada laikā vai piecas tonsilīta paasinājuma epizodes gada laikā divus gadus pēc kārtas, vai trīs tonsilīta paasinājuma epizodes gada laikā trīs gadus pēc kārtas, ir ieteicama tonsilektomija. Tonsilīta recidīvu etiopatoģenēze nav skaidra un tiek aktīvi pētīta. Neefektīvas antibakteriālās terapijas iemesls tiek skaidrots ar baktēriju specifiskiem rezistences mehānismiem, biofilmu veidošanos, antibiotiku nepietiekamu koncentrāciju aukslēju mandeļu audos. RT samazina pacientu dzīves kvalitāti, ir medicīnisks un finansiāls slogs. RT paasinājumi var komplicēties par paratonsilāru abscesu (PTA).

Pētījuma mērķis bija identificēt RT un PTA etioloģiskos aģentus aukslēju mandeļu kriptās un parenhīmā, noteikt patogēno baktēriju antibakteriālo rezistenci un spēju veidot biofilmas un izvērtēt to nozīmi RT un PTA patoģenēzē un klīniskajā norisē.

Mērķa sasniegšanai tika izveidots pētījuma plāns. Pētījumā tika iekļautas vairākas sadaļas. 1. sadaļas uzdevums bija izvērtēt RT un PTA pacientus, kuriem veikta tonsilektomija. RT grupu veidoja 99 plānveida pacienti, kuriem tonsilektomija veikta plānveida kārtā sakarā ar tonsilīta recidīviem, neskatoties uz atbilstošu antibakteriālo terapiju. PTA pacientu grupu veidoja 29 pacienti, kuriem veikta akūta tonsilektomija PTA drenāžai. 2. sadaļas uzdevums bija izvērtēt aukslēju mandeļu kriptu paraugu precīzāko iegūšanas metodi. Tika izveidota RT pacientu grupa, kas sastāvēja no 25 pacientiem. Šajā sadaļā aukslēju mandeļu kriptu paraugi iegūti divos veidos un salīdzināti iegūtie rezultāti. 3. sadaļas uzdevums bija izvērtēt veselo indivīdu aukslēju mandeļu kriptu mikrobioloģiskos rezultātus. Tika izveidota veselo indivīdu grupa un iekļauts 91 cilvēks – Medicīnas fakultātes studenti bez aukslēju mandeļu patoloģijas. 4. sadaļas uzdevums bija veikt bakterioloģisku novērtēšanu pacientiem pēc tonsilektomijas. Grupā tika iekļauti 16 pacienti, kuriem pirms gada bija RT un tika veikta tonsilektomija. Šiem pacientiem tika veikta klīniska un bakterioloģiska novērtēšana. Katrā sadaļā iekļautajiem pacientiem tika ņemti aukslēju mandeļu kriptu paraugi bakterioloģiskai analīzei, antibakteriālās jutības un biofilmu producēšanas spēju analīzei. Izmeklējumi veikti Rīgas Stradiņa universitātes Bioloģijas un mikrobioloģijas katedras laboratorijā un Paula Stradiņa klīniskās universitātes slimnīcas Bakterioloģijas laboratorijā.

Pētījuma gaitā tika izstrādāta un patentēta biopsijas adata aukslēju mandeļu kriptu optimāla parauga iegūšanai. Pētījumā konstatēts, ka RT pacientiem, salīdzinot ar PTA pacientiem un veseliem indivīdiem, bija lielāka mikroorganismu daudzveidība aukslēju mandeļu kriptās, dominējošais patogēns bija *S. aureus*. RT, PTA pacientiem tika konstatēti biofilmu veidojošie celmi, bet nozīmīga antibakteriālā rezistence netika konstatēta un netika novērota saistība starp biofilmas producēšanas fenotipu un antibakteriālo jutību. Veseliem indivīdiem bez aukslēju mandeļu patoloģijas no aukslēju mandeļu kriptām tika izdalīti klīniski nozīmīgi patogēni (*S. aureus, K. pneumoniae, P. aeruginosa*) un jutīgi biofilmu producējoši celmi. Veselo indivīdu aukslēju mandeļu kriptas ir nozīmīgs patogēno baktēriju un biofilmu producējošo celmu rezervuārs. Mūsu pētījuma rezultāti apliecina, ka tonsilektomija ir efektīva bakteriālās kolonizācijas novēršanai.

Promocijas darbs tika veidots kā rakstu kopa, kas apkopo pētījuma rezultātus. Promocijas darbā iegūtie rezultāti par RT un orālo mikrobiotu ir ar starptautisku nozīmi un satur rekomendācijas izmantošanai klīniskajā praksē.

Atslēgvārdi: zinātnes nozare – medicīna, apakšnozare – otorinolaringoloģija, recidivējošs tonsilīts, paratonsilārs abscess, biofilmas, antibakteriālā jutība.

Abstract

Role of Biofilms in Pathogenesis and Clinical Course of Recurrent Tonsillitis and Peritonsillar Abscess

After nasopharyngitis and otitis media tonsillitis is the 3rd most common infection that requires patients to see an otorhinolaryngologist. When episodes of tonsillitis recur, acute tonsillitis diagnosis turns into recurrent tonsillitis (RT). Exacerbations of RT are treated with antibiotics. Currently, the frequency of exacerbations in the last 3 years is used as the main determining factor in the selection of patients for elective tonsillectomy. If there are 7 episodes of tonsillitis in a year, or 5 episodes of tonsillitis in a year for 2 consecutive years, or 3 episodes of tonsillitis in a year for 3 consecutive years, tonsillectomy is recommended. The etiopathogenesis of RT is not clear and is being actively studied. The reason for ineffective antibacterial therapy is explained by specific resistance mechanisms of bacteria, formation of biofilms, insufficient concentration of antibiotics in tonsillar tissue. RT reduces patients` quality of life and is a medical and a financial burden. During exacerbation of RT a peritonsillar abscess (PTA) as a complication may develop.

Therefore, the aim of the study was to identify the etiological agents of RT and PTA in tonsil crypts and parenchyma samples, to determine antibacterial resistance and biofilm formation capacity of pathogenic bacteria, and to evaluate their importance in the pathogenesis and clinical course of RT and PTA.

The study included several parts: 1st part was dedicated for evaluating RT and PTA patients undergoing tonsillectomy. The RT group consisted of 99 patients who underwent planned tonsillectomy due to RT despite appropriate antibacterial therapy. The PTA patient group consisted of 29 patients who underwent acute tonsillectomy for drainage of peritonsillar abscess. The objective of 2nd part was to evaluate the most accurate method of obtaining samples from tonsillar crypts. The study group of 25 patients with RT was created. In this part, tonsillar crypt samples were obtained in 2 ways and the results were compared. The objective of 3rd part was to evaluate the microbiological results of tonsillar crypt sample of healthy individuals. A group of healthy individuals without tonsillar pathology consisted of 91 participants – students of Medical Faculty of Rīga Stradiņš University. In 4th part a bacteriological evaluation of post-tonsillectomy patients was performed. The group consisted of 16 patients who previously had RT and had a tonsillectomy performed a year ago. These patients were subjected to clinical and bacteriological evaluation. During each part tonsillar crypt samples were taken for bacteriological analysis, antibacterial sensitivity and biofilm production capacity of isolated strains was assessed. The examinations were carried out in the

laboratory of the Biology and Microbiology Department of Rīga Stradiņš University and the Laboratory of Bacteriology of the Pauls Stradiņš Clinical University Hospital.

During the study a biopsy needle was developed and patented for obtaining an optimal sample of tonsillar crypts. The study found that RT patients, compared to PTA patients and healthy individuals, had a higher diversity of microorganisms in the tonsillar crypts, the predominant pathogen being *S. aureus*. Biofilm forming strains were detected in RT, PTA patients, but no significant antibacterial resistance was detected and no relationship between the biofilm producing phenotype and antibacterial sensitivity was observed. Clinically relevant pathogens (*S. aureus*, *K. pneumoniae*, *P. aeruginosa*) and susceptible biofilm producing strains were isolated from tonsillar crypts of healthy individuals without tonsillar pathology. The tonsillar crypts of healthy individuals are an important reservoir of pathogenic bacteria and biofilm producing strains. The results of our study confirm that tonsillectomy is effective in preventing bacterial colonization.

The thesis is presented as a unifying material of peer-review publications, it summarizes the results of the research. The obtained results on RT and oral microbiota are of international importance and contain recommendations for use in clinical practice.

Keywords: sector – medicine, sub-sector – otorhinolaryngology, recurrent tonsillitis, peritonsillar abscess, biofilms, antibacterial susceptibility.

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Darbā izmantotie saīsinājumi

Abreviatūra	Nosaukums angļu valodā	Nosaukums latviešu valodā	Nosaukums latīņu valodā
Ae			*Acinetobacter ewoffi*
Aju			*Acinetobacter junii*
Ajo			*Acinetobacter johnsoni*
A. johnsoni			*Acinetobacter johnsoni*
AK	*Amikacin*	Amikacīns	
AMP	*Ampicillin*	Ampicilīns	
AMS	*Ampicillin/sulbactam*	Ampicilīns/sulbaktāms	
Ap			*Acinetobacter pitti*
A. pitti			*Acinetobacter pittii*
AUG	*Amoxicillin/clavulanic acid*	Amoksicilīns/klavulānskābe	
ASO	*Antistreptolysin O*	Antistreptolizīns O	
BP	*Benzylpenicillin*	Benzilpenicilīns	
C	*Chloramphenicol*	Hloramfenikols	
C. albicans			*Candida albicans*
CAZ	*Ceftazidime*	Ceftazidīms	
CD	*Climdamycin*	Klindamicīns	
CIP	*Ciprofloxacin*	Ciprofloksacīns	
CRP	*C-reactive protein*	C reaktīvais proteīns	
CRO	*Ceftriaxone*	Ceftriaksons	
CTX	*Cefotaxime*	Cefotaksīms	
D	*Nasal vestibule*	Deguna priekštelpa	*Vestibulum nasi*
E	*Erythromycin*	Eritromicīns	
ESBL	*Extended spectrum beta-lactamase*	Paplašināta spektra beta-laktamāze	
EUCAST	*European Committee on Antimicrobial Susceptibility Testing*	Eiropas Antimikrobiālās jutības noteikšanas komisija	
ETP	*Ertapenem*	Ertapenēms	
FOX	*Cefoxitin*	Cefoksitīns	
GM	*Gentamicin*	Gentamicīns	
H. influenzae			*Haemophilus influenzae*
HIV	*Human immunodeficiency virus*	Cilvēka imūndeficīta vīruss	
I	*Intermediate resistant*	Jutīgs paaugstinātā koncentrācijā	
IMP	*Imipenem*	Imipenēms	
J	*Sensitive*	Jutīgs	
K	*Crypt*	Kripta	

Kp			*Klebsiella pneumoniae*
K. pneumoniae			*Klebsiella pneumoniae*
LB	*Luria-Bertani broth*	Lurija-Bertani buljons	
LEU	*White blood cells*	Leikocīti	
LEV	*Levofloxacin*	Levofloksacīns	
MALDI-TOF MS	*Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry*	Ar matricas asistētā lāzera desorbcijas/jonizācijas lidojuma laika masas spektometrija	
MEM	*Meropenem*	Meropenēms	
MRSA	*Methicillin-resistant Staphylococcus aureus*	Meticilīna rezistentais *Staphylococcus aureus*	
MSSA	*Methicillin-sensitive Staphylococcus aureus*	Meticilīna jutīgais *Staphylococcus aureus*	
NK	*Negative control*	Negatīvā kontrole	
NOR	*Norfloxacin*	Norfloksacīns	
N. subflava			*Neisseria subflava*
OB	*Optical density*	Optiskais blīvums	
OBr	*Optical density`s cut-off value*	Optiskā blīvuma robežvērtība	
P	*Armpit*	Paduse	
PSKUS	*Pauls Stradiņš Clinical University Hospital*	Paula Stradiņa klīniskās universitātes slimnīca	
Pa			*Pseudomonas aeruginosa*
P. aeruginosa			*Pseudomonas aeruginosa*
P. intermedia			*Prevotella intermedia*
PTA	*Peritonsillar abscess*	Paratonsilārs abscess	
R	*Resistant*	Rezistents	
RSU	*Rīga Stradiņš University*	Rīgas Stradiņa universitāte	
RT	*Recurrent tonsillitis*	Recidivējošs tonsilīts	
S	*Female*	Sieviete	
Sa			*Staphylococcus aureus*
S. aureus			*Staphylococcus aureus*
S. agalactiae			*Streptococcus agalactiae*
S. anginosus			*Streptococcus anginosus*
S. dysgalactiae			*Streptococcus dysgalactiae*
S. epidermidis			*Staphylococcus epidermidis*
S. pneumoniae			*Streptococcus pneumoniae*

S. pyogenes			*Streptococcus pyogenes*
S. viridans			*Streptococcus viridans*
SKI	*Interquartile range*	Starpkvartiļu intervāls	
SN	*Standard deviation*	Standartnovirze	
Spp.	*Species*	Sugas	
Sl			*Serratia liquefaciens*
S. liquefaciens			*Serratia liquefaciens*
SXT	*Trimethoprim/sulfamethoxazole*	Trimetoprims/sulfametoksazols	
SV	*International unit*	Starptautiskā vienība	
TE	*Tonsillectomy*	Tonsilektomija	
TZP	*Piperacillin/tazobactam*	Piperacilīns/tazobaktāms	
TSB	*Tryptic Soy Broth*	Triptozes sojas buljons	
V	*Male*	Vīrietis	
Ž	*Throat*	Rīkle	

Ievads

Recidivējošs tonsilīts (RT) ir aukslēju mandeļu recidivējošs iekaisums, ko izraisa pārsvarā vai vienīgi baktērijas (Zautner et al., 2010; Cavalcanti et al., 2019; Buname et al., 2021). Tonsilīta paasinājuma epizodes raksturo paaugstināta ķermeņa temperatūra, sāpes rīklē un sāpīga rīšana, žāvas apsārtums, aukslēju mandeļu pietūkums ar vai bez aplikuma un kakla limfadenopātija (Buname et al., 2021). Starp tonsilīta paasinājumu epizodēm var saglabāties diskomforts rīklē, detrīta pastiprināta krāšanās aukslēju mandeļu kriptās, halitoze un kakla limfadenopātija (bin Abu Bakar et al., 2018). RT diagnozi nosaka klīniski, balstoties uz anamnēzes datiem (Windfuhr et al., 2016a; Sykes et al., 2020). RT diagnozi var noteikt, ja 12 mēnešu periodā ir vairāk nekā divas atsevišķas tonsilīta epizodes (Burton et al., 2014). Tonsilīta paasinājumu laikā ārstēšanā lieto antibiotikas (Windfuhr et al., 2016a; Katkowska et al., 2017). Tonsilektomija ir indicēta pacientiem ar RT, ja ir septiņas tonsilīta paasinājuma epizodes gada laikā vai piecas tonsilīta paasinājuma epizodes gada laikā divus gadus pēc kārtas, vai trīs tonsilīta paasinājuma epizodes gada laikā trīs gadus pēc kārtas (Paradise et al., 1984; Sykes et al., 2020).

Iekaisums no aukslēju mandeļu audiem var izplatīties uz blakusesošiem paratonsilāriem audiem un izveidot abscesu paratonsilārā telpā (Windfuhr et al., 2016a; Peter Sell et al., 2023). Paratonsilārs abscess (PTA) ir tonsilīta biežākā strutainā komplikācija (Klug, 2017). PTA diagnozi nosaka, ņemot vērā anamnēzes un pacienta apskates datus. Pacientam ar PTA raksturīgs drudzis, vienpusējas sāpes rīklē, neskaidra runa, dažreiz trisms, kakla descendējošo limfmezglu iesaiste iekaisuma procesā (Klug, 2017). Apskatē PTA raksturīgs vienpusējs gļotādas apsārtums un palpatori blīvs un sāpīgs pietūkums paratonsilārā un piegulošo mīksto aukslēju rajonā (Slouka et al., 2020). PTA visbiežāk ir vienpusējs. PTA ārstēšanā ir nepieciešama antibakteriālā terapija un ķirurģiska ārstēšana – abscesa incīzija un drenāža vai tonsilektomija akūtā kārtā (Windfuhr et al., 2016b; Klug, 2017). Vēl joprojām nav skaidri iemesli, kādēļ konservatīvā terapija mēdz būt neefektīva.

RT epizodes samazina dzīves kvalitāti, ir finansiāli zaudējumi darbnespējas un medicīnas izdevumu dēļ (Windfuhr et al., 2016b; Tzelnick et al., 2020). Atkārtotu antibakteriālās terapijas kursu risks ir antibakteriālās rezistences veicināšana. Ķirurģiskās ārstēšanas un anestēzijas lietošanā ir tādi potenciālie riski kā asiņošana, asiņu aspirācija, blakusesošo struktūru ievainojums, ilgstoša brūču dzīšana u.c. (Windfuhr et al., 2016b).

Nesekmīgas antibakteriālās terapijas iemesli ir vairāki, piemēram, grūtības identificēt tonsilītu izraisošās baktērijas, zema antibiotiku koncentrācija aukslēju mandeļu audos, patogēna saglabāšanās intracelulāri epitēlija vai makrofāgiem līdzīgās antigēnprezentējošās šūnās,

iesaistīto patogēnu specifiski antibakteriālās rezistences mehānismi vai biofilmu veidošanās (Pichichero & Casey, 2007; Zautner et al., 2010; bin Abu Bakar et al., 2018). Ir veikti vairāki pētījumi, kas raksturo RT un PTA etioloģijā iesaistīto patogēnu spektru, etioloģija bieži ir polimikrobiāla (Zautner et al., 2010; Klug, 2017). Aukslēju mandeļu paraugu mikrobioloģisko analīzi un etioloģiskā ierosinātāja identificēšanu apgrūtina liela mikroorganismu daudzveidība no normālās orālās mikrobiotas (Windfuhr et al., 2016a; Dickinson et al., 2020). Aukslēju mandelēs ir lielākā mikrobiālā daudzveidība ar nozīmīgām individuālām atšķirībām (Aas et al., 2005; Jorgensen et al., 2015; Ivaska et al., 2020). Aukslēju mandeļu virsmu klāj normālā orālā mikrobiota, kas parasti nav iesaistīta aukslēju mandeļu iekaisumu etioloģijā, kaut gan autoinfekcija ar orālo mikrobiotu ir iespējama (Windfuhr et al., 2016a; Haq et al., 2017). Vietās ar augstu bakteriālo kolonizāciju ir jāizvērtē izdalīto baktēriju klīniskā un etioloģiskā nozīme (Vaikjärv et al., 2016). Aukslēju mandeļu iekaisumu etioloģijā prevalē aukslēju mandeļu parenhīmā vai kriptās mītošās baktērijas, nevis virsmā esošās baktērijas (Khadilkar & Ankle, 2016). Aukslēju mandeļu kriptas ir šauras, zarotas ejas aukslēju mandeļu audos. Mikrobioloģiskai analīzei paraugi no aukslēju mandeļu kriptām tiek uzskatīti par precīzākiem un atbilstošākiem salīdzinājumā ar aukslēju mandeļu virsmas uztriepēm (Haq et al., 2017; Dickinson et al., 2020).

RT pacientu aukslēju mandeļu audos ir augsta *Staphylococcus aureus* (*S. aureus*) prevalence, *S. aureus* tiek uzskatīts par RT galveno etioloģisko faktoru (Brook & Foote, 2006; Zautner et al., 2010; Katkowska et al., 2017; Kostić et al., 2022). Tomēr nav skaidra šī patogēna loma RT paasinājumu patoģenēzē, abscesu veidošanā un antibakteriālās rezistences veidošanā. Mikrobioloģisko analīzi apgrūtina *S. aureus* asimptomātiska nēsāšana (Chmielowiec-Korzeniowska et al., 2020). *S. aureus* spēj iegūt plaša spektra antibakteriālo rezistenci, tomēr RT pētījumos *S. aureus* neuzrāda augstu antibakteriālo rezistenci un tiek pētīti citi aizsardzības mehānismi, piemēram, biofilmu veidošanās (bin Abu Bakar et al., 2018; Cavalcanti et al., 2019; Katkowska et al., 2020; Kostić et al., 2022). *Klebsiella pneumoniae* (*K. pneumoniae*) noturīgu biofilmu veidošanas spējas ir apstiprinātas pētījumos. Pateicoties *K. pneumoniae* biofilmām, tās spēj kolonizēt elpceļus, aizdeguni, mandeles (Alasil et al., 2013; Wang et al., 2020).

Tiek uzskatīts, ka RT patoģenēzē ir iesaistītas biofilmas veidojošās baktērijas (Kostić et al., 2022). Pētījumos, izmantojot skenējošo elektronmikroskopu, konstatēta statistiski nozīmīga biofilmu klātbūtne RT pacientu grupā salīdzinājumā ar kontroles grupu, attiecīgi 80 % (16/20) un 45 % (9/20) (Woo et al., 2012). Baktērijas, kas atrodas daudzslāņainā, nobriedušā biofilmā, ir pasargātas no saimniekorganisma imūnās sistēmas aizsargmehānismiem – makrofāgiem un antivielām, kā arī tās nesasniedz antibiotikas (Archer et al., 2011; Lister & Horswill, 2014; Hamilos, 2019). Biofilmu īpašības izskaidro patogēnu

spēju izdzīvot aukslēju mandeļu audos, neskatoties uz atkārtotiem antibakteriālās terapijas kursiem (Archer et al., 2011; Lister & Horswill, 2014; Moormeier & Bayles, 2017). RT paasinājumus izskaidro ar brīvo planktonisko baktēriju klātbūtni, bet hroniskos simptomus starp tonsilīta paasinājuma epizodēm, ar kriptās un biofilmu kolonijās esošām baktērijām, kuras ilgstoši spēj rezistēt saimniekorganisma imūnajai aizsargsistēmai (bin Abu Bakar et al., 2018).

Pētījuma mērķis

Identificēt RT un PTA etioloģiskos aģentus aukslēju mandeļu kriptās un parenhīmā, noteikt to antibakteriālo rezistenci un spēju veidot biofilmas un izvērtēt to nozīmi RT un PTA patoģenēzē un klīniskajā norisē.

Darba uzdevumi

Promocijas darba mērķa sasniegšanai izvirzīti šādi uzdevumi:

1. Izdalīt un identificēt RT un PTA pacientu aukslēju mandeļu kriptās esošos mikroorganismus, izvērtēt izdalīto *S. aureus* un *K. pneumoniae* biofilmu veidošanas spēju, antibakteriālo jutību un to lomu tonsilīta recidīvu un PTA attīstībā.
2. Salīdzināt baktēriju klātbūtni RT pacientu aukslēju mandeļu virsmas un aukslēju mandeļu kriptu materiālā, izvērtēt to lomu RT etioloģijā.
3. Identificēt un raksturot nozīmīgāko rīkles un elpceļu patogēnu klātbūtni veselu indivīdu aukslēju mandeļu kriptās.
4. Izvērtēt un salīdzināt *S. aureus* kolonizācijas biežumu un klīnisko nozīmi RT pacientiem tonsilektomijas materiālā un *S. aureus* nēsāšanu vienu gadu pēc operācijas.

Darba hipotēze

RT un PTA etioloģiskie ierosinātāji lokalizējas aukslēju mandeļu kriptās vai parenhīmā, nevis mandeļu virsmā, un RT un PTA klīniskajā norisē būtiska nozīme ir baktēriju veidam un dažādībai, to biofilmas veidošanas spējai un antibakteriālai rezistencei.

Darba novitāte

Veikta plaša RT un PTA etioloģisko ierosinātāju identifikācija, izmantojot *MALDI-TOF MS* un *VITEK-2 Compact* iekārtu. Optimālai un precīzai aukslēju mandeļu kriptu parauga iegūšanai izgatavota, patentēta un pirmo reizi izmantota punktēšanas biopsijas adata (Klagisa et al., 2021a). Veikts veselo indivīdu aukslēju mandeļu mikrobiotas pētījums. Izvērtēta biofilmu veidojošo izolātu klātbūtne RT un PTA pacientiem un izvērtēta to saistība ar antibakteriālo

rezistenci. Pirmo reizi veikts RT pacientu monitorings gadu pēc tonsilektomijas, izvērtēta ķirurģiskās terapijas efektivitāte.

Personīgais ieguldījums

Darba autore veikusi visu pētījumā iesaistīto veselo indivīdu un pacientu apskati, atlasi, konsultāciju, ārstēšanu un operācijas, ņēmusi aukslēju mandeļu biopsijas un ieguvusi pacientu materiālu bakterioloģiskai izvērtēšanai, pati personīgi veikusi visu paraugu bakterioloģisko izmeklēšanu (mikroorganismu izdalīšanu un identifikāciju, antimikrobiālās jutības un biofilmu veidošanas noteikšanu), analizējusi iegūtos datus un sagatavojusi materiālus biopsijas adatas patenta ieguvei (patents Nr.: LVP2020000055).

Ētiskie aspekti

Pētījums veikts saskaņā ar Helsinku deklarāciju, un pētījumu apstiprinājusi Rīgas Stradiņa universitātes Ētikas komiteja 2017. gada 30. novembrī, atļauja Nr. 49/30.11.2017., un 2020. gada 10. septembrī, atļauja Nr. 6-1/09/22 (sk. 6. pielikumu). Visi pētījumā iekļautie pacienti un veselie brīvprātīgie parakstīja informētas piekrišanas veidlapu līdzdalībai pētījumā.

1. Materiāli

Prospektīvā pētījumā, iekļaujot RT un PTA pacientus un veselos indivīdus (sk. 1.1. attēlu), veikta medicīniskā apskate, apkopoti dati par viņu veselību, aukslēju mandeļu iekaisumiem, epizodēm un to biežumu, lietoto antibakteriālo terapiju. Veikts aukslēju mandeļu mikrobioloģiskais izvērtējums: identificēta mikroorganismu daudzveidība, izvērtēta to antibakteriālā jutība un spēja veidot biofilmas, salīdzināts aukslēju mandeļu paraugu iegūšanas veids – ar punkcijas metodi no aukslēju mandeļu kriptām un iztriepe no aukslēju mandeļu virsmas. Izvērtēta *S. aureus* sastopamība pacientiem vienu gadu pēc tonsilektomijas. Aukslēju mandeļu paraugi iegūti Paula Stradiņa klīniskās universitātes slimnīcas (PSKUS) Otorinolaringoloģijas klīnikā. Mikrobioloģiskie pētījumi veikti Rīgas Stradiņa universitātes Bioloģijas un mikrobioloģijas katedras laboratorijā un PSKUS Bakterioloģijas laboratorijā.

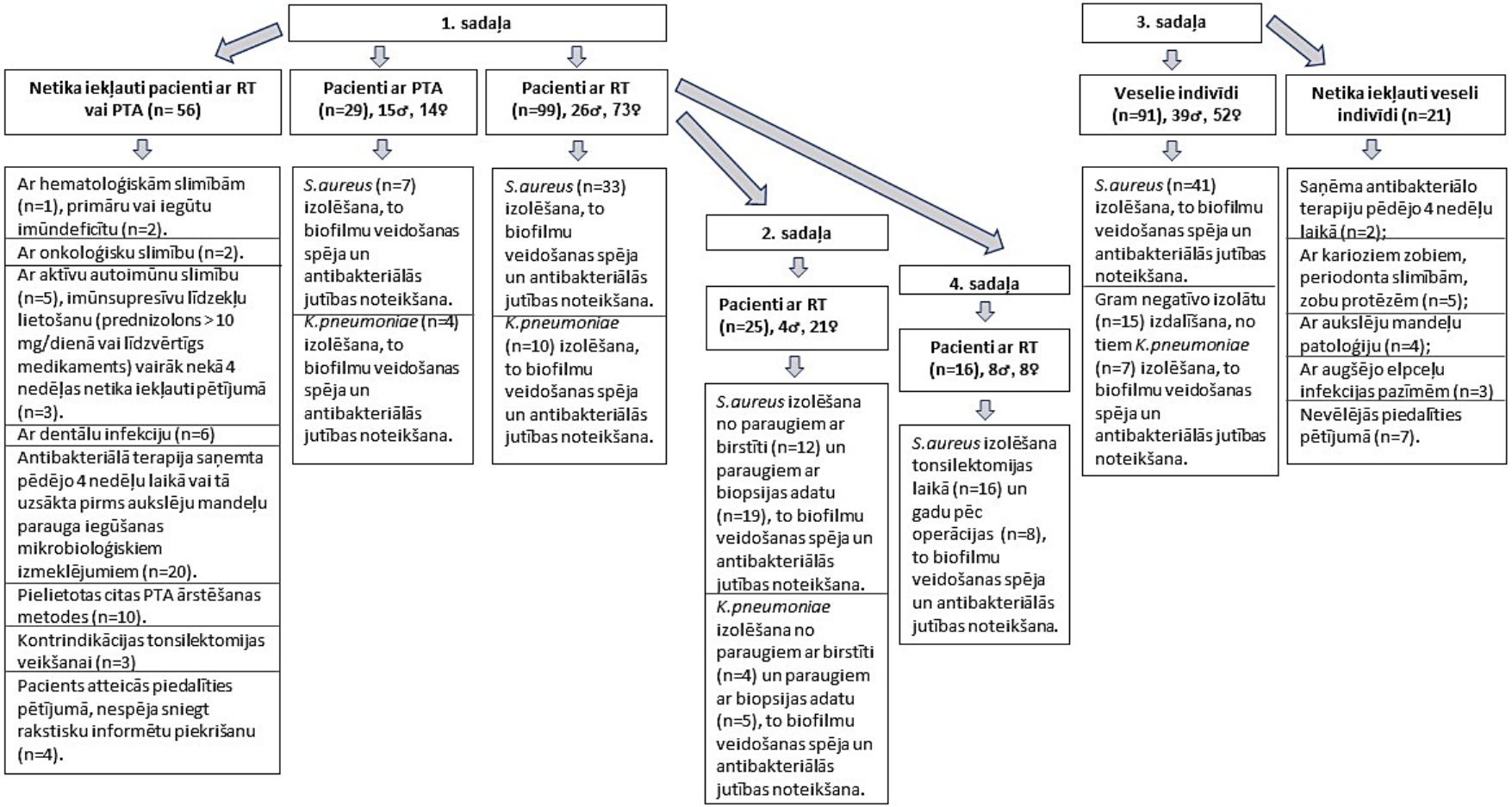
RT un PTA pacienti tika iekļauti pētījumā laika posmā no 2018. līdz 2020. gadam, kad saņēma terapiju PSKUS Otorinolaringoloģijas klīnikā. Iekļaušanas kritēriji bija šādi: RT vai PTA diagnoze, saņemta ķirurģiska ārstēšana (abpusēja tonsilektomija), nav saņemta antibakteriālā terapija pēdējo četru nedēļu laikā. RT definēts atbilstoši *Paradise* kritērijiem kā septiņas tonsilīta epizodes gada laikā, piecas tonsilīta epizodes gada laikā divus gadus pēc kārtas vai trīs tonsilīta epizodes gadā trīs gadus pēc kārtas, neskatoties uz saņemto atbilstošo antibakteriālo terapiju (Paradise et al., 1984; Windfuhr et al., 2016a, 2016b). PTA tika diagnosticēts klīniski, balstoties uz pacientu sūdzībām un apskates datiem: paratonsilārā rajona un mīksto aukslēju vienpusējs, palpatori blīvs un sāpīgs piemilzums ar žāvas gļotādas hiperēmiju, dažreiz trismu, kakla limfadenopātiju, kakla sāpēm, neskaidru runu, drudzi. RT un PTA abscesa diagnoze noteikta PSKUS pēc sertificēta otorinolaringologa konsultācijas.

Visiem pacientiem ar RT tika veikta abpusēja tonsilektomija plānveida kārtā un pēdējā tonsilīta paasinājuma epizode ne agrāk kā četras nedēļas pirms operācijas. Visiem PTA pacientiem tika veikta abpusēja tonsilektomija akūtā kārtā tonsilīta paasinājuma laikā.

Pētījumā netika iekļauti pacienti ar hematoloģiskām slimībām (trombocitopēniju vai koagulopātiju), primāru vai iegūtu imūndeficītu, ar dentālu infekciju, ja antibakteriālā terapija saņemta pēdējo četru nedēļu laikā vai tā uzsākta pirms aukslēju mandeļu parauga iegūšanas mikrobioloģiskiem izmeklējumiem, ja lietotas citas PTA ārstēšanas metodes, piemēram, PTA incīzija un drenāža, vai bija kontrindikācijas tonsilektomijas veikšanai (kontrindikācijas operācijai vai vispārējai anestēzijai), vai pacients atteicās piedalīties pētījumā, nespēja sniegt rakstisku informētu piekrišanu. Pacienti ar onkoloģisku slimību, aktīvu autoimūnu slimību, imūnsupresīvu līdzekļu lietošanu (prednizolons > 10 mg/dienā vai līdzvērtīgs medikaments) vairāk nekā četras nedēļas netika iekļauti pētījumā. Par pacientu blakusslimībām informācija

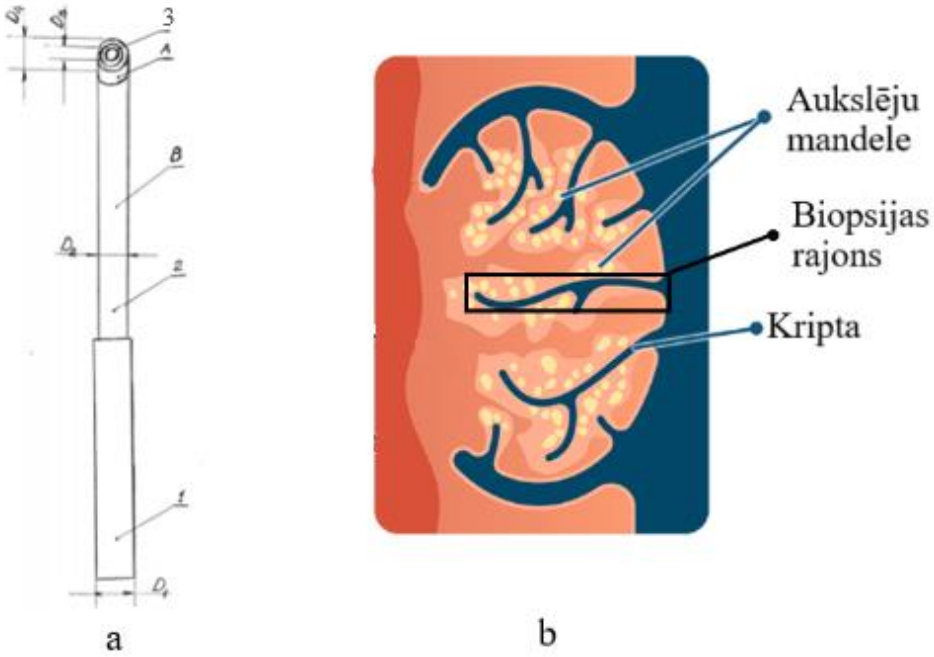
iegūta no ģimenes ārsta nosūtījuma, jo otorinolaringologs ir sekundārās veselības aprūpes speciālists, un pēc sertificēta otorinolaringologa un anesteziologa konsultācijas PSKUS.

Pētījumā iekļautie veselie indivīdi bija Rīgas Stradiņa universitātes Medicīnas fakultātes studenti. Studenti iesaistīti pētījumā no 2019. gada 1. oktobra līdz 31. decembrim, kad apmeklēja otorinolaringoloģijas mācību ciklu. Pētījumā iekļautie studenti bija bez blakusslimībām, bez aukslēju mandeļu patoloģijas, bez augšējo elpceļu infekcijas pazīmēm pētījuma norises laikā un nebija saņēmuši antibakteriālo terapiju vismaz četras nedēļas līdz dalībai pētījumā, balstoties uz studentu sniegto informāciju par viņu veselības stāvokli. Pētījumā netika iekļauti studenti, kuri pirms dalības pētījumā saņēmuši antibakteriālo terapiju pēdējo četru nedēļu laikā, ar karioziem zobiem, periodonta slimībām, zobu protēzēm, balstoties uz studentu sniegto informāciju par viņu veselības stāvokli, vai kuri nevēlējās piedalīties pētījumā.



1.1. attēls. **Pētījuma shēma**

Paraugi mikrobioloģiskai analīzei tika iegūti šādi: ar sterilu birstīti no aukslēju mandeļu kriptām (*Kito brush*, references numurs 0640, Kaltek srl, Padova, Itālija) (2. un 3. sadaļa); ar sterilu punktēšanas biopsijas adatu aukslēju mandeļu audu fragments ar kriptu un tās saturu tonsilektomijas laikā (1., 2., 4. sadaļa); ar sterilu vates kociņu no deguna priekštelpas (latīniski *vestibulum nasi*), padusēm un aukslēju mandeļu nišām pēc tonsilektomijas (4. sadaļa). Aukslēju mandeļu audu fragmenta iegūšanai pētniecības nolūkos tika izstrādāta punktēšanas biopsijas adata ar pagarinātu, liektu rokturi un apļveida asmeni, kura izmēri piemēroti optimāla lieluma parauga iegūšanai (sk. 1.2. attēlu), patents Nr.: LVP2020000055 (Klagisa et al., 2021a).



1.2. attēls. **Biopsijas adatas (a) un aukslēju mandeles biopsijas rajona (b) shēma**

Punktēšanas adata sastāv no asmeņa (3) un turētāja (2) ar divām daļām (A) un (B), kas savā starpā veido 125° leņķi.

Iegūtie paraugi tika ievietoti *Amies* transporta barotnēs un 24 stundu laikā istabas temperatūrā nogādāti uz laboratoriju.

2. Metodes

2.1. Mikroorganismu identifikācija

Iegūtie paraugi aerobos apstākļos tika kultivēti 36 ± 1 °C 24–48 h uz Kolumbijas asins agara, Mannīta sāls agara, *MacConcey* agara un *Sabouraud* dekstrozes agara barotnēm (*Liofilchem*, Itālija). Anaerobu mikroorganismu kultivēšanai izmantota Brucellas asins agara barotne anaerobā maisiņu sistēmā, kas inkubēta 36 ± 1 °C līdz piecām diennaktīm. *Streptococcus pneumoniae* kultivēšanai izmantota Kolumbijas asins agara barotne ar optohina disku, kas inkubēta 36 ± 1 °C 24–48 h ar CO_2 indikatoru. *Haemophylus* spp. kultivēšanai izmantota šokolādes agara barotne, kas inkubēta CO_2 inkubatorā 36 ± 1 °C 24–48 h ar oleandomicīna disku. Izdalītām tīrkultūrām veikta krāsošana pēc Grama un mikroskopēšana. Mikroorganismu identifikācija veikta, izmantojot *Microflex LT* (*Bruker Daltonics flexAnalysis* 3.4 versija, *Bruker Daltonics GmbH & Co.* KG, Brēmene, Vācija) ar matricas asistētā lāzera desorbcijas/jonizācijas lidojuma laika masas spektometra (*MALDI-TOF MS*) sistēmu vai izmantojot iekārtu *VITEK-2 Compact* (*BioMérieux, Marcy-l'Étoile*, Francija).

Normālā orālā mikrobiota tika definēta atbilstoši Eiropas Klīniskās mikrobioloģijas un infekcijas slimību biedrības nostādnēm (Cornaglia & Courcol, 2012).

2.2. Biofilmu noteikšana, izmantojot kristālvioleto metodi

Izdalītie grampozitīvie izolāti tika iesēti triptozes sojas buljonā (TSB) ar 1 % glikozi, gramnegatīvie izolāti tika iesēti Lurija-Bertani (LB) buljonā, inkubēti 37 °C 16–18 h. Uzsētie TSB un LB buljoni atšķaidīti ar sterilu TSB vai LB buljonu attiecībā 1:100. Tad 150 µL atšķaidītā buljona sterilā manierē ar multikanālu pipeti pārnesti sterilā 96 bedrīšu platē (*Thermo Scientific™ Nunc MicroWell 96-Well Microplates, flat bottom, Thermo Fisher Scientific*, Roskilde, Dānija). Katrā platē bija 11 izolāti un viena negatīvā kontrole (sterils buljons), astoņas bedrītes katram izolātam, katrs eksperiments veikts trijās platēs. Uzsētās plates inkubētas aerobos apstākļos 37 °C h. Pēc inkubācijas visas plašu bedrītes tika iztukšotas, neizmantojot pipeti, saudzīgi izlejot šķidrumu klīnisko atkritumu maisā. Katra plates bedrīte tika izskalota trīs reizes ar sterilu 250 µL 0,9 % fizioloģiskā šķīduma. Pēc mazgāšanas veikta krāsošana ar kristālvioleto šķīdumu, katrā bedrītē pievienojot 150 µL 0,1 % kristālvioletā šķīduma. Pēc 15 minūtēm krāsa tika saudzīgi izlieta un katra bedrīte mazgāta trīs reizes ar 250 µL destilēta ūdens. Beigās katrai bedrītei pievienoja 150 µL 96 % etanola. Pēc tam ar mikroplates spektrofotometru tika izmērīti bedrīšu optiskie blīvumi (OB), izmantojot 570 nm viļņa garumu (*Tecan Infinite F50*, Mannedorfa, Šveice, ar *Magellan™* lasītāja vadības un datu analīzes programmatūru V 6.6) (Reisner et al., 2006).

2.3. Biofilmu aprēķins

Tika aprēķināta katra izolāta OB vidējās vērtības un izteiktas skaitļos. Optiskā blīvuma robežvērtība (OBr) tika definēta kā trīs standartnovirzes (SN) virs negatīvās kontroles vidējā OB, un tā tika aprēķināta katrai platei atsevišķi. Izolāti tika sadalīti šādi: OB ≤ OBr = biofilmas neveidojošs izolāts, OBr < OB ≤ 2 × OBr = vājas biofilmas veidotājs, 2 × OBr < OB≤ 4 × OBr = mērenas biofilmas veidotājs un 4 × OBr < OB = stingras biofilmas veidotājs (Stepanović et al., 2007).

2.4. Antibakteriālās jutības noteikšana

Antibakteriālās jutības testēšana tika veikta ar *Kirby-Bauer* disku difūzijas metodi. Vienu nakti kultivētas kultūras tika suspendētas fizioloģiskā šķīdumā līdz 0,5 *McFarland* vienībām (*McFarland Densitometer DEN-1, Biosan*, Latvija). Suspensijas tika vienmērīgi inokulētas uz *Mueller-Hinton* agara (*Oxid*, Lielbritānija) virsmas, izmantojot sterilu vates kociņu. Uz inokulētām agara barotnēm tika novietoti standartizēti antimikrobiālo vielu noteiktā koncentrācijā saturoši diski. *S. aureus* izolātiem izmantoti šādi diski: cefoksitīns 30 µg, ceftriaksons 30 µg, benzilpenicilīns 1 SV, ampicilīns 2 µg, ampicilīns/sulbaktāms 10 µg/10 µg, amoksicilīns/klavulānskābe 20 µg/10 µg, norfloksacīns 10 µg, amikacīns 30 µg, eritromicīns 15 µg, klindamicīns 2 µg un hloramfenikols 30 µg (*Liofilchem*, Itālija). *K. pneumoniae* un *Serratia liquefaciens* (*S. liquefaciens*) izolātiem izmantoti šādi diski: amoksicilīns/klavulānskābe 20 µg/10 µg, piperacilīns/tazobaktāms 30 µg/6 µg, cefotaksīms 5 µg, ceftazidīms 10 µg, ertapenēms 10 µg, imipenēms 10 µg, meropenēms 10 µg, ciprofloksacīns 5 µg, gentamicīns 10 µg un trimetoprims/sulfametoksazols 1,25 µg/23,75 µg (*Liofilchem*, Itālija). *Acinetobacter* spp. izolātiem izmantoti šādi diski: imipenēms 10 µg, amikacīns 30 µg, gentamicīns 10 µg, trimetoprims/sulfametoksazols 1,25 µg/23,75 µg, ciprofloksacīns 5 µg un levofloksacīns 5 µg (*Liofilchem*, Itālija). Plates inkubētas 16–18 h, 36 ± 1 °C. Pēc inkubācijas tika izmērīts baktēriju audzes aiztures zonas diametrs ap katru no antibiotiku diskiem atbilstoši Eiropas Antimikrobiālās jutības noteikšanas komisijas (*EUCAST*) rekomendācijām *Clinical breakpoints and dosing of antibiotics* (Versija 10.0, Janvāris 2020) (Anonymous, 2020).

3. Statistiskā analīze

Ar *Kolmogorov-Smirnov* testu tika pārbaudīta datu atbilstība normālsadalījumam. Normāli sadalītu datu centrālās tendences raksturotas, izmantojot vidējās vērtības ar SN, savukārt normālam sadalījumam neatbilstoši dati raksturoti, izmantojot mediānās vērtības ar starpkvartiļu intervālu (SKI).

Atšķirības starp grupām tika noteiktas, izmantojot:

1) neatkarīgo izlašu *Kruskal-Wallis* testu (piemēram, vecuma atšķirības starp pacientiem RT un PTA grupās, atšķirības starp *S. aureus* četru izolātu biofilmu veidošanās spējām);
2) *Pearson* χ^2 testu (piemēram, sakarības starp grampozitīvu mikrobu klātbūtni un biofilmu veidošanas spēju);
3) *Fisher* eksakto testu (piemēram, sakarības starp *S. aureus, K. pneumoniae* identifikāciju birstītes un punktēšanas biopsijas adatas paraugos, sakarības starp tonsilīta recidīvu skaitu un blakusslimībām; tonsilīta recidīvu skaitu un *S. aureus* klātbūtni aukslēju mandeļu biopsijas paraugos vai *K. pneumoniae* klātbūtni aukslēju mandeļu biopsijas paraugos; tonsilīta epizožu skaitu un *S. aureus* biofilmas veidošanas pakāpi vai *K. pneumoniae* biofilmas veidošanas pakāpi; tonsilīta epizožu skaitu un *S. aureus* antibakteriālo rezistenci vai *K. pneumoniae* antibakteriālo rezistenci);
4) *Mann-Whitney* U testu (piemēram, atšķirības starp *S. aureus* divu izolātu biofilmu veidošanās spējām);
5) *McNemar* testu (metožu salīdzināšana, sakarības starp *K. pneumoniae* un *S. aureus* identifikāciju birstītes un punktēšanas biopsijas paraugos).

Rezultātus uzskatīja par statistiski ticamiem, ja būtiskuma līmeņa (p) vērtība bija < 0,05. Statistiskā analīze tika veikta, izmantojot *IBM SPSS Statistics* versiju 26 (Čikāga, IL, ASV) un *Microsoft Excel 10* (*Microsoft, Redmond*, Vašingtona, ASV).

4. Rezultāti

4.1. Mikroorganismu kolonizācijas, biofilmu veidošanas un antibakteriālās jutības analīze recidivējoša tonsilīta un paratonsilārā abscesa pacientiem

Pētījums ir aprakstīts Renātas Klagišas, Kārļa Rāceņa, Renāra Broka, Artas Olgas Balodes, Ligijas Ķīses un Jutas Kroičas rakstā *Analysis of Microorganism Colonization, Biofilm Production, and Antibacterial Susceptibility in Recurrent Tonsillitis and Peritonsillar Abscess Patients. International Journal of Molecular Sciences*. 2022, 23, 10273. doi.org/10.3390/ijms231810273

4.1.1. Pacientu dati

Šajā pētījumā tika iekļauti 128 pacienti, no tiem 29 pacientiem tika diagnosticēts PTA, 99 pacientiem – RT. Pētījuma pacientiem laika posmā no 2018. līdz 2020. gadam tika veikta tonsilektomija PSKUS. RT pacientu grupā tika iekļauti 26 vīrieši (26 %) un 73 (74 %) sievietes vecuma diapazonā no 20 līdz 72 gadiem ar vidējo vecumu 32,94 gadi (± 11,19). PTA pacientu grupā tika iekļauti 15 (52 %) vīrieši un 14 (48 %) sievietes vecuma diapazonā no 18 līdz 58 gadiem ar vidējo vecumu 32,4 gadi (± 12,2). Pacientu vecuma un dzimuma attiecības ir norādītas 4.1. tabulā.

4.1. tabula

Pētāmās populācijas raksturojums

Parametrs		RT	PTA	p vērtība
Dzimums	Vīrieši, n (%)	26 (26 %)	15 (52 %)	p = 0,061
	Sievietes, n (%)	73 (74 %)	14 (48 %)	p = 0,061
Vecums	Vecums (intervāls, vidējais ± SN), gadi	20–72, 32,94 ± 11,19	18–58, 32,4 ± 12,2	p = 0,279
	Vecums (mediāna, SKI), gadi	31, 10	31, 16	–
Laboratoriskie rādītāji	CRP, mediāna, mg/L	1,17	85,5	p < 0,001
	LEU, mediāna, × 10^9/L	6,52	12,97	p < 0,001

SN – standartnobīde; SKI – starpkvartiļu intervāls; CRP – C reaktīvais proteīns; LEU – leikocīti; RT – recidivējošs tonsilīts; PTA – paratonsilārs abscess.

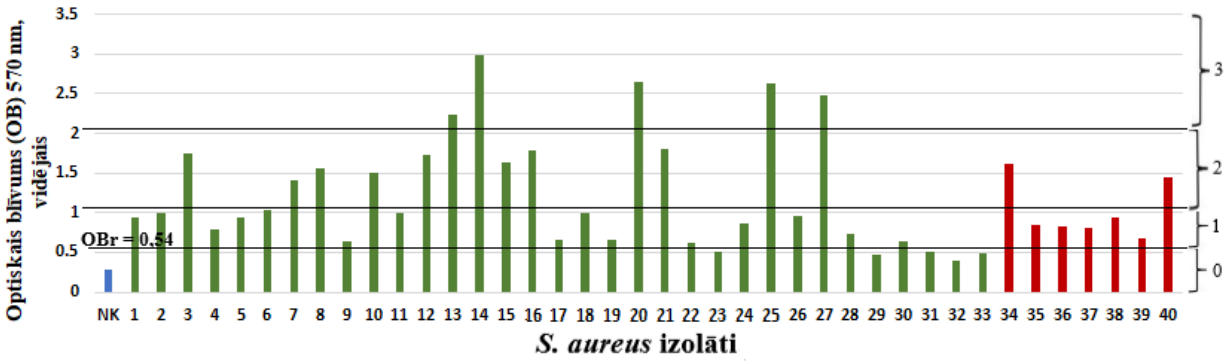
Saskaņā ar mūsu datiem RT grupā dominēja sievietes, savukārt PTA grupā dzimumu attiecība bija līdzsvarotāka (sk. 4.1. tabulu). Vecuma atšķirības starp pacientiem RT un PTA grupās nebija (neatkarīgo izlašu *Kruskal-Wallis* tests, p = 0,617). Pacientiem ar PTA tika konstatēts paaugstināts leikocītu skaits (LEU) un C reaktīvā proteīna (CRP) līmenis asins paraugos, un pacientiem ar RT bija LEU un CRP līmenis normas robežās. Šīs atšķirības bija statistiski nozīmīgas.

4.1.2. Izolēto mikroorganismu daudzveidība

No 128 pārbaudītajiem pacientu paraugiem pozitīvs kultivēšanas rezultāts (vismaz 1 patogēns vai potenciālais patogēns) tika atklāts 60 pacientiem (60,6 %) RT grupā un 24 pacientiem (82,8 %) PTA grupā (*Pearson* χ^2 tests, $p = 0,027$). Vislielākā mikroorganismu daudzveidība tika konstatēta pacientiem ar RT. Kultivēšanas rezultāts bija negatīvs, t. i., tika atrasta tikai normālā orālā mikrobiota 39 pacientiem (39,4 %) RT grupā un 5 pacientiem (17,2 %) PTA grupā (sk. 1. un 2. tabulu 5. pielikumā). Neatkarīgi no pacientu grupas visbiežāk izdalītā patogēnā baktērija bija *S. aureus*, kas tika izolēta kā vienīgais mikroorganisms vai kopā ar citiem potenciāli patogēniem mikroorganismiem (sk. 1. un 2. tabulu 5. pielikumā). RT grupā *S. aureus* tika izolēts 33 no 99 (33,3 %) gadījumiem, un PTA grupā tika izolēts 7 no 29 (24,14 %) gadījumiem. Grampozitīvās baktērijas dominēja, bet vismaz viena gramnegatīva baktērija tika atklāta 22 no 99 (22,2 %) pacientiem RT grupā un 8 no 29 (27,6 %) pacientiem PTA grupā. Visizplatītākā gramnegatīvā baktērija bija *K. pneumoniae*; tā tika izolēta 10 no 99 (10,1 %) RT gadījumiem un 4 no 29 (13,4 %) PTA gadījumiem (sk. 1. un 2. tabulu 5. pielikumā). Tika izolētas *Candida* sugas, un tās dominēja pacientiem ar PTA, kur tās konstatētas 14 no 29 (48,3 %) gadījumiem un pārsvarā kā monokultūras (*Fisher* tests, $p < 0,001$).

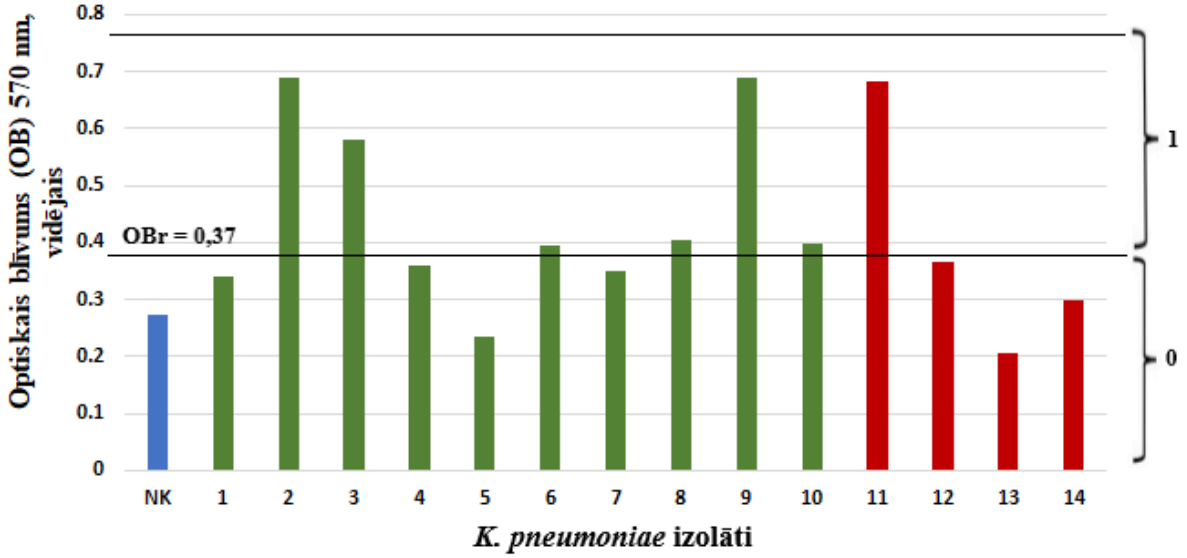
4.1.3. Biofilmas

Vismaz 1 biofilmu veidojošs izolāts tika konstatēts 37 no 99 (37,4 %) RT un 8 no 29 (27,6 %) PTA gadījumiem (*Pearson* χ^2 tests, $p = 0,332$). Mērenas vai stingras biofilmas veidojoši izolāti tika konstatēti 16 no 37 RT gadījumiem un 2 no 8 PTA gadījumiem. RT grupā starp 33 *S. aureus* izolātiem 5 bija stingras, 8 bija mērenas un 15 bija vājas biofilmas veidotāji, bet 5 biofilmas neveidoja vispār. PTA grupā no 7 *S. aureus* izolātiem 2 bija vājas biofilmas veidotāji un 5 bija biofilmas neražojoši izolāti. RT grupā no 10 *K. pneumoniae* izolātiem 6 bija vājas biofilmas veidotāji un 4 bija biofilmas neveidojoši izolāti. PTA grupā no 4 *K. pneumoniae* izolātiem 1 bija vājš biofilmas veidojošs un 3 bija biofilmas neveidojoši izolāti. Visu *S. aureus* un *K. pneumoniae* izolātu biofilmu vidējie optiskie blīvumi (OB) ir apkopoti 4.1. un 4.2. attēlā.



4.1. attēls. ***S. aureus* izolātu biofilmas veidošanās spēja uz mikroplatēm pacientiem ar RT un pacientiem ar PTA**

Zaļie stabiņi (1–33) apzīmē RT pacientus. Sarkanie stabiņi (34–40) apzīmē pacientus ar PTA. Stabiņi attēlo OB vidējās vērtības (mērīts pie 570 nm viļņa garuma). TSB ar 1 % glikozi izmantots kā negatīvā kontrole (NK, zilais stabiņš). Ar horizontālām līnijām ir atzīmēta OB robežvērtība (OBr) un biofilmas veidošanas līmeņi: 0 – biofilmas neražotāji; 1 – vāji biofilmas ražotāji; 2 – mērenas biofilmas ražotāji; 3 – stingras biofilmas ražotāji.



4.2. attēls. ***K. pneumoniae* izolātu biofilmas veidošanas spēja uz mikroplatēm pacientiem ar RT un pacientiem ar PTA**

Zaļie stabiņi (1–10) apzīmē RT pacientus. Sarkanie stabiņi (11–14) apzīmē pacientus ar PTA. Stabiņi attēlo OB vidējās vērtības (mērītas pie 570 nm viļņa garuma). LB barotne kā negatīva kontrole (NK, zilais stabiņš). Ar horizontālām līnijām ir atzīmēta robežvērtība (OBr) un biofilmas veidošanas līmeņi: 0 – biofilmas neražotāji; 1 – vājas biofilmas ražotāji.

Tika konstatēta statistiski nozīmīga sakarība starp grampozitīvu baktēriju klātbūtni un biofilmas veidojošo fenotipu RT grupā un PTA grupā. Ja klātesošs ir grampozitīvs mikrobs, visticamāk, būs biofilmu veidojošs fenotips (*Pearson* χ^2 tests, $p < 0{,}001$). Netika konstatētas statistiski nozīmīgas sakarības starp gramnegatīviem mikrobiem, *Candida* spp. klātbūtni, blakusslimībām, tonsilīta epizodēm vai PTA slimības vēsturē un biofilmu veidojošo celmu RT vai PTA grupā. Lai gan netika konstatētas statistiski nozīmīgas sakarības starp biofilmu veidojošo izolātu klātbūtni vai *S. aureus* biofilmu veidojošo izolātu klātbūtni un pacientu grupu, PTA grupā bija mazāk biofilmu veidojošo variantu, salīdzinot ar RT grupu (sk. 4.2. tabulu).

4.2. tabula

Pacientu mikrobioloģisko datu salīdzinājums RT un PTA grupā

Pacientu mikrobioloģiskie dati		RT grupa	PTA grupa	p vērtība
Izdalīšanas biežums	*S. aureus*, n (%)	33/99 (33,33 %)	7/29 (24,14 %)	p = 0,347
	K. pneumoniae, n (%)	10/99 (10,10 %)	4/29 (13,79 %)	p = 0,519
	Candida spp., n (%)	8/99 (8,08 %)	14/29 (48,28 %)	p < 0,001
Biofilmas, vidējais OB	*S. aureus* biofilmas, vidējais OB	1,24	1,02	p = 0,929
	K. pneumoniae biofilmas, vidējais OB	0,44	0,39	p = 0,322
Biofilmas veidojoši izolāti	Biofilmas veidojoši izolāti, n	37	8	p = 0,111
	S. aureus biofilmas veidojoši izolāti, n	28	7	p = 0,642
	S. aureus mērenu un stingru biofilmu veidojoši izolāti, n (%)	13/33 (39,39 %)	2/7 (28,57 %)	p = 0,691
	K. pneumoniae mērenu un stingru biofilmu veidojoši izolāti, n	0	0	–
Sakarības starp mainīgajiem pa grupām	Grampozitīvs mikrobs un biofilmu veidojošs izolāts	p < 0,001	p < 0,001	–
	Gramnegatīvs mikrobs un biofilmu veidojošs izolāts	p = 0,227	p > 0,999	–
	Candida spp. un biofilmu veidojošs izolāts	p > 0,999	p = 0,215	–
	Blakusslimības un biofilmu veidojošs izolāts	p = 0,759	p = 0,540	–
	Tonsilītu epizodes un biofilmu veidojošs izolāts	p = 0,313	p = 0,738	–
	PTA slimības vēsturē un biofilmu veidojošs izolāts	p = 0,091	p = 0,640	–

4.1.4. Antibakteriālā jutība

S. aureus izolāti bija jutīgi pret cefoksitīnu, ceftriaksonu, ampicilīnu/sulbaktāmu, amoksicilīnu/klavulānskābi, amikacīnu, eritromicīnu, klindamicīnu un hloramfenikolu, jutīgi pret ciprofloksacīnu paaugstinātā koncentrācijā, bet rezistenti pret benzilpenicilīnu un ampicilīnu. *S. aureus* 1 izolāts tika identificēts kā meticilīna rezistentais *S. aureus* (MRSA), jo bija rezistents pret cefoksitīnu. *S. aureus* izolāti, kas bija rezistenti pret benzilpenicilīnu, ampicilīnu un vismaz vienu citu antibiotiku, attēloti 4.3. un 4.4. tabulā kopā ar biofilmas veidošanas spēju. Rezistentie izolāti pārsvarā bija biofilmu neveidojoši vai vājas biofilmas ražotāji. Neviens no *K. pneumoniae* izolātiem nebija paplašināta spektra beta-laktamāzes ražotājs.

4.3. tabula

S. aureus antibakteriālā jutība un biofilmu veidošanas spēja

Antibiotikas	RT pacientu _S. aureus_ izolāti (n = 33)			PTA pacientu _S. aureus_ izolāti (n =7)		
	Rezistentie izolāti (n)	Biofilmas neveidojoši vai vājas biofilmas veidojoši izolāti (n)	Mērenu vai stingru biofilmu veidojoši izolāti (n)	Rezistentie izolāti (n)	Biofilmas neveidojoši vai vājas biofilmas veidojoši izolāti	Mērenu vai stingru biofilmu veidojoši izolāti
BP, AMP, CIP*	20/33	12/20	8/20	5/7	4/5	1/5
BP, AMP, CIP*, CD*	1/33	1	–	–	–	–
CIP*	9/33	5/9	4/9	2/7	1/2	1/2
BP, AMP, CIP*, E	1/33	–	1	–	–	–
CIP*, E	1/33	1	–	–	–	–
FOX, CRO, BP, AMP, AMS, AUG, CIP*	1**/33	1	–	–	–	–

FOX, cefoksitīns; CRO, ceftriaksons; BP, benzilpenicilīns; AMP, ampicilīns; AMS, ampicilīns/sulbaktāms; AUG, amoksicilīns/klavulānskābe; CIP, ciprofloksacīns; E, eritromicīns; CD, klindamicīns. * Jutīgs paaugstinātā koncentrācijā; ** MRSA; katra antibiotiku rezistence tika noteikta atsevišķi.

4.4. tabula

_S_. aureus izolātu antibakteriālā jutība un biofilmu veidošanas spējas pa pacientu grupām

Pacientu grupa	Biofilmu veidošanas spēja	Antibakteriālā rezistence pret BP un AMP	Antibakteriālās rezistences nav vai rezistence tikai pret vienu antibiotiķi	p vērtība
RT grupa	Biofilmu neveido vai ir vāja biofilma	14	6	p = 0,590
	Mērena vai stingra biofilma	9	4	p > 0,999
PTA grupa	Biofilmu neveido vai ir vāja biofilma	4	1	p > 0,999
	Mērena vai stingra biofilma	1	1	p > 0,999
PTA + RT grupa	Biofilmu neveido vai ir vāja biofilma	18	7	p = 0,590
	Mērena vai stingra biofilma	10	5	p > 0,999

BP, benzilpenicilīns; AMP, ampicilīns. Katra antibiotiku rezistence tika noteikta atsevišķi.

Kopsavilkums. RT un PTA gadījumā biežāk tika izdalīts *S. aureus*, 33,3 % RT pacientu un 24,14 % PTA pacientu. *K. pneumoniae* izdalīja salīdzinoši retāk – attiecīgi 10,1 % un 13,4 %. Biofilmu veidojošie izolāti RT gadījumā sasniedza 37,4 % un PTA gadījumā 27,6 %. *S. aureus* RT un PTA gadījumā ir jutīgi pret empīrisku antibakteriālu terapiju, ar izteiktu jutību pret amoksicilīnu ar klavulānskābi un klindamicinīnu. *S. aureus* 1 izolāts tika identificēts kā MRSA.

4.2. Uz aukslēju mandeļu virsmas un aukslēju mandeļu kriptās esošo patogēno mikroorganismu novērtējums pacientiem ar recidivējošu tonsilītu

Pētījums ir aprakstīts Renātas Klagišas, Jutas Kroičas un Ligijas Ķīses rakstā *S. aureus and K. pneumoniae on the Surface and within Core of Tonsils in Adults with Recurrent Tonsillitis. Medicina.* 2021, 57(10), 1002; https://doi.org/10.3390/medicina57101002

Šajā pētījumā tika iekļauti 25 pieauguši pacienti, kuriem diagnosticēts RT un kuriem no 2020. gada augusta līdz 2020. gada septembrim tika veikta tonsilektomija PSKUS. Pētījumā iekļautie pacienti bija 4 vīrieši un 21 sieviete vecuma diapazonā no 20 līdz 71 gadam ar mediāno vecumu 31 gads. No Rīgas bija 17 pacienti, no Jelgavas 3 pacienti, no citām Latvijas pilsētām (Engures, Bauskas, Salaspils, Limbažiem un Tukuma) 5 pacienti.

S. aureus tika identificēts ar birstīti ņemtos paraugos 12 gadījumos un ar punktēšanas biopsijas adatas paraugos 9 gadījumos. *Fisher* eksaktais tests uzrādīja statistiski nozīmīgu sakarību starp *S. aureus* identifikāciju no birstītes un punktēšanas biopsijas adatas paraugos ($p = 0,004$). *K. pneumoniae* tika identificēta ar birstīti ņemtos paraugos 4 gadījumos un 5 gadījumos ar punktēšanas biopsijas adatas paraugos. *Fisher* eksaktais tests uzrādīja statistiski nozīmīgu sakarību starp *K. pneumoniae* identifikāciju birstītes un punktēšanas biopsijas paraugos ($p < 0,001$). *McNemar* tests, kas lietots, lai salīdzinātu divu metožu precizitāti, statistiski nozīmīgas atšķirības neuzrādīja.

S. aureus, kas izdalīti no punktēšanas biopsijas adatas paraugiem, tika noteikta biofilmu veidošanas spēja (sk. 4.5. tabulu). No 9 *S. aureus* izolātiem 5 bija biofilmu veidojoši, 2 izolāti bija stingru biofilmu veidotāji, 3 izolāti bija vāju biofilmu veidotāji, bet 4 no 9 *S. aureus* izolātiem biofilmu neveidoja.

No 5 *K. pneumoniae* izolātiem 4 bija vāju biofilmu veidotāji un 1 no 5 *K. pneumoniae* bija biofilmu neveidojošs izolāts (sk. 4.5. tabulu).

4.5. tabula

Ar punktēšanas biopsijas adatu tonsilektomijas laikā iegūto *S. aureus* un *K. pneumoniae* biofilmu veidošanas un antibakteriālās jutības raksturojums

Izolāts	Biofilmas		Antibakteriālā jutība			
	Biofilmas veidojoši izolāti (n)	Biofilmas neveidojoši izolāti (n)	BP	AMP	CIP	E
S. aureus	5 (9)	4 (9)	R 7 (9)	R 7 (9)	I 9 (9)	0 (9)
K. pneumoniae	4 (5)	1(5)	R 5 (5)	R 5 (5)	0 (5)	R 5 (5)

BP, benzilpenicilīns; AMP, ampicilīns; CIP, ciproflokasacīns; E, eritromicīns, R, rezistents; I, jutīgs paaugstinātā koncentrācijā.

S. aureus un *K. pneumoniae* izolātiem, kas identificēti punktēšanas biopsijas adatu paraugos, tika noteikta antibakteriālā jutība. No 9 *S. aureus* izolātiem 7 bija rezistenti pret benzilpenicilīnu un ampicilīnu; 5 no 7 izolātiem bija biofilmu veidotāji. Visi *S. aureus* izolāti bija jutīgi uz ampicilīnu/sulbaktāmu, amiksicilīnu/klavulānskābi, amikacīnu, eritromicīnu, klindamicīnu, hloramfenikolu un uz ciprofloksacīnu paaugstinātā koncentrācijā. Visi *K. pneumoniae* izolāti bija rezistanti uz benzilpenicilīnu, ampicilīnu un eritromicīnu, bet jutīgi uz ampicilīnu/sulbaktāmu, amiksicilīnu/klavulānskābi, piperacilīnu/tazobaktāmu, ceftazidīmu, cefotaksīmu, ceftriaksonu, meropenēmu, imipenēmu, amikacīnu un ciprofloksacīnu.

Nebija statistiski nozīmīgas sakarības starp tonsilīta recidīvu skaitu un blakusslimībām (*Fisher* eksaktais tests, p = 0,542); tonsilīta recidīvu skaitu un *S. aureus* klātbūtni aukslēju mandeļu biopsijas paraugos (*Fisher* eksaktais tests, p = 0,260) vai *K. pneumoniae* klātbūtni aukslēju mandeļu biopsijas paraugos (*Fisher* eksaktais tests, p > 0,999); tonsilīta epizožu skaitu un *S. aureus* biofilmas veidošanas pakāpi (*Fisher* eksaktais tests, p = 0,238) vai *K. pneumoniae* biofilmas veidošanas pakāpi (*Fisher* eksaktais tests, p = 0,617); tonsilīta epizožu skaitu un *S. aureus* antibakteriālo rezistenci (*Fisher* eksaktais tests, p = 0,294) vai *K. pneumoniae* antibakteriālo rezistenci (*Fisher* eksaktais tests, p > 0,999) (sk. 4.6. tabulu).

4.6. tabula

Pētāmās pacientu grupas raksturojums

N.p.k.	Vecums	Dzimums	Dzīvesvieta	RT	Sa birstīte	Sa biopsija	Kp birstīte	Kp biopsija	Sa biofilma, pakāpe	Kp biofilma, pakāpe	Sa rezistence (BP, AMP)	Kp rezistence (BP, AMP, E)
1	31	S	Rīga	+	+	+	–	–	3		R	
2	27	S	Engure	+	+	+	–	–	1		R	
3	30	S	Bauska	+	–	–	+	+		1		R
4	30	S	Rīga	+	–	+	–	–	3		R	
5	20	S	Jelgava	+	–	–	–	–				
6	64	S	Jelgava	–	+	–	–	–				
7	31	S	Rīga	–	–	–	–	–				
8	26	S	Rīga	+	+	+	–	–	1		R	
9	32	S	Rīga	+	–	–	–	–				
10	36	S	Rīga	+	+	+	–	–	0		R	
11	31	S	Rīga	+	–	–	–	–				
12	26	S	Salaspils	+	+	+	–	–	1		R	
13	31	V	Rīga	+	–	–	–	+		0		R
14	25	S	Rīga	+	–	–	+	+		1		R
15	71	S	Rīga	+	+	–	–	–				
16	61	S	Jelgava	+	–	–	–	–				
17	55	V	Rīga	–	–	–	+	+		1		
18	34	S	Limbaži	+	–	–	–	–				
19	22	S	Rīga	+	+	+	–	–	0			

4.6. tabulas turpinājums

N.p.k.	Vecums	Dzimums	Dzīvesvieta	RT	Sa birstīte	Sa biopsija	Kp birstīte	Kp biopsija	Sa biofilma, pakāpe	Kp biofilma, pakāpe	Sa rezistence (BP, AMP)	Kp rezistence (BP, AMP, E)
20	55	S	Rīga	–	–	–	–	–				
21	40	S	Rīga	+	–	–	–	–				
22	38	V	Tukums	+	+	+	+	+	0	1		
23	27	V	Rīga	+	+	+	–	–	0			
24	41	S	Rīga	+	+	–	–	–				
25	58	S	Rīga	+	+	–	–	–				

RT, recidivējoša tonsilīta epiozedes pēdējos 3 gados; 0, biofilmu neveidojošs izolāts; 1, vāju biofilmu veidojošs izolāts; 2, vidēju biofilmu veidojošs izolāts; 3, stingru biofilmu veidojošs izolāts; R, rezistents; BP, bezilpenicilīns; AMP, ampicilīns; E, eritromicīns.

Tonsilektomijas operācijas materiāls tika nosūtīti histopatoloģiskai izmeklēšanai. Visiem pacientiem histopatoloģiskā izmeklējuma slēdziens bija hronisks nespecifisks tonsilīts.

Kopsavilkums. No aukslēju mandeļu virsmas iegūtais materiāls ir ar zemāku ticamību, jo ir bagātīgi kontaminēts ar orālo mikrobiotu. Biežākie RT ierosinātāji *S. aureus* un *K. pneumoniae* tika izdalīti gan no aukslēju mandeļu virsmas, gan no aukslēju mandeļu kriptām. No kriptām izdalītās grampozitīvās (5 no 9) un gramnegatīvās (4 no 5) baktērijas bija biofilmas veidojošie izolāti.

4.3. No veselu indivīdu aukslēju mandeļu kriptām iegūto patogēno mikroorganismu biofilmu veidošanas, antibakteriālās jutības novērtējums

Pētījums ir aprakstīts Renātas Vīksnes, Kārļa Rāceņa, Renāra Broka, Artas Olgas Balodes, Ligijas Ķīses un Jutas Kroičas rakstā *In Vitro Assessment of Biofilm Production, Antibacterial Resistance of Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter spp. Obtained from Tonsillar Crypts of Healthy Adults. Microorganisms*. 2023; 11(2):258. https://doi.org/10.3390/microorganisms11020258

4.3.1. Pacientu dati

Pētījuma grupā tika iekļautas 52 sievietes (57 %) un 39 vīrieši (43 %) vecumā no 19 līdz 29 gadiem (vidēji 21,2 ± 1,41 gads, mediānais vecums 21 gads).

4.3.2. Izolēto mikroorganismu daudzveidība

No 91 dalībnieka paraugiem pozitīvs kultivēšanas rezultāts (vismaz 1 patogēns vai potenciālais patogēns) tika konstatēts 54 dalībnieku paraugos (59,3 %) (sk. 4.7. tabulu).

4.7. tabula

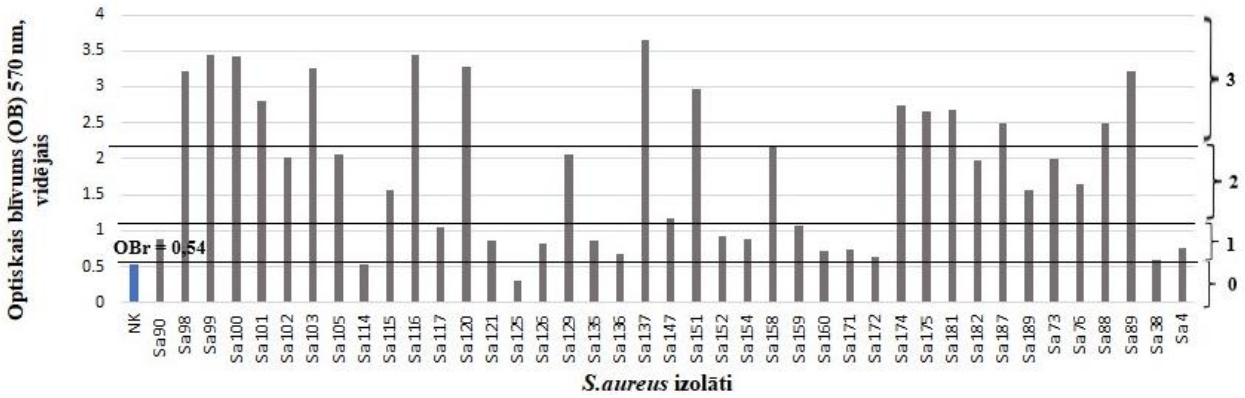
Mikroorganismi, kas izolēti no 91 vesela indivīda aukslēju mandeļu kriptām

Izolētu baktēriju kombinācijas	Skaits (n)
Normālā orālā mikrobiota	37
S. aureus + normālā orālā mikrobiota	20
S. aureus	16
S. aureus + *A. junii*	2
S. aureus + *K. pneumoniae*	1
S. aureus + *Candida* spp. + *S. viridans*	1
S. aureus + *K. pneumoniae* + *S. liquefaciens* + normālā orālā mikrobiota	1
K. pneumoniae	5
P. aeruginosa	2
A. pittii	2
A. johnsonii	1
S. liquefaciens	1
S. dysgalactiae	1
A. ewofii + normālā orālā mikrobiota	1

Kultivēšanas rezultāts bija negatīvs, t. i., bija tikai normālā orālā mikrobiota, 37 dalībnieku paraugos (40,7 %) (sk. 4.7. tabulu). Visbiežāk izolētā patogēnā baktērija bija *S. aureus*, kas tika izolēta kā vienīgais mikroorganisms vai kopā ar citiem potenciāli patogēniem mikroorganismiem 41 dalībnieka paraugā (45 %) (sk. 4.7. tabulu). Dominēja grampozitīvas baktērijas, bet vismaz 1 gramnegatīva baktērija tika konstatēta 16 paraugos (17,6 %). Starp gramnegatīvajām baktērijām *K. pneumoniae* bija visizplatītākā, un tā tika izolēta 7 paraugos.

4.3.3. Biofilmas

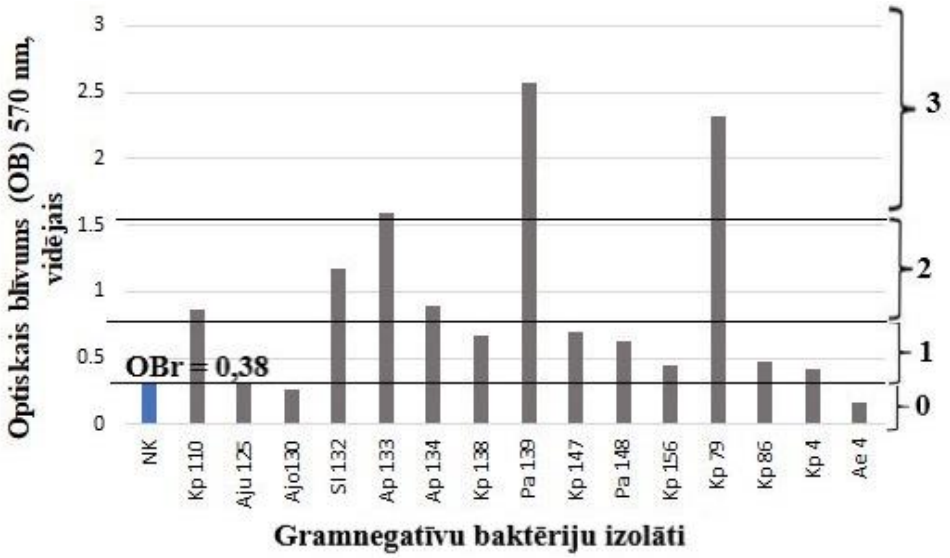
Biofilmu veidošanas spēja tika novērota 41 *S. aureus* izolātam un 15 gramnegatīvu baktēriju izolātiem. *S. aureus* pārsvarā bija biofilmas veidojoši varianti: 25 no 41 (61 %) *S. aureus* izolāta bija vidēji vai spēcīgi biofilmas veidotāji, un 14 no 41 (34,1 %) *S. aureus* izolāta bija vāji biofilmas veidotāji, bet 2 no 41 (4,9 %) biofilmas neveidoja (sk. 4.3. attēlu).



4.3. attēls. ***S. aureus* 41 izolāta biofilmas veidošanas spēja uz mikroplatēm**

Stabiņi attēlo OB vidējās vērtības (mērītas pie 570 nm viļņa garuma). TSB ar 1 % glikozi izmantots kā negatīvā kontrole (NK). Numurs apzīmē dalībnieku; burti norāda izolātu: Sa – *Staphylococcus aureus*. Ar horizontālām līnijām ir atzīmēta robežvērtība (OBr) un biofilmas veidošanas līmeņi: 0 – biofilmas neražotāji; 1 – vāji biofilmas ražotāji; 2 – mērenas biofilmas ražotāji; 3 – stingras biofilmas ražotāji.

Gramnegatīvo baktēriju vidū 6 no 15 (40 %) baktērijām bija vidējas vai spēcīgas biofilmas ražotājas un 6 no 15 (40 %) izolātiem bija vāji biofilmas ražotāji, bet 3 no 15 (20 %) izolātiem biofilmas neveidoja (sk. 4.4. attēlu).



4.4. attēls. **Gramnegatīvu baktēriju 15 izolātu biofilmas ražošanas spēja uz mikroplatēm**

Stabiņi attēlo OB vidējās vērtības (mērītas pie 570 nm viļņa garuma). LB barotne izmantota kā negatīva kontrole (NK). Numurs apzīmē dalībnieku; burti norāda izolātu: Kp – *Klebsiella pneumoniae*; Aju – *Acinetobacter junii*; Ajo – *Acinetobacter johnsoni*; Sl – *Serratia liquefaciens*; Ap – *Acinetobacter pitti*; Pa – *Pseudomonas aeruginosa*; Ae – *Acinetobacter ewoffi*. Robežvērtība (OBr) un biofilmas ražošanas līmeņi ir atzīmēti ar horizontālām līnijām: 0 – biofilmas neražotāji; 1 – vāji biofilmas ražotāji; 2 – mēreni biofilmas ražotāji; 3 – spēcīgi biofilmas ražotāji.

Pētījuma dalībnieku mikrobioloģisko datu kopsavilkums ir parādīts 4.8. tabulā. Bija statistiski nozīmīga saistība starp grampozitīvu baktērijas klātbūtni un biofilmas veidošanas fenotipu. Ja ir grampozitīvs mikrobs, visticamāk, būs biofilmas veidojošais fenotips (*Pearson* χ^2 tests, $p < 0{,}001$) (sk. 4.8. tabulu).

4.8. tabula

Pētījuma dalībnieku mikrobioloģisko datu apkopojums

Mikrobioloģiskie dati		Rezultāts	p vērtība
Izdalīšanas biežums	Tikai normālā orālā mikrobiota, n (%)	37/91 (40,7 %)	–
	Grampozitīvi izolāti, n	43	–
	Gramnegatīvi izolāti, n	17	–
Biofilma, vidējais OB	*S. aureus* biofilmas, vidējais OB	1,89	–
	Gramnegatīvu mikrobu biofilmas, vidējais OB	0,95	–
Biofilmu veidojoši celmi	Biofilmu veidojoši izolāti, n	51	–
	S. aureus biofilmu veidojoši izolāti, n	39	–
	Gramnegatīvu mikrobu biofilmu veidojoši izolāti, n	12	–
	Stingru un mērenu biofilmu veidojoši izolāti, n	31	–
Sakarības starp mainīgajiem	Grampozitīvs mikrobs un biofilmu veidojošs izolāts	–	p < 0,001
	Gramnegatīvs mikrobs un biofilmu veidojošs izolāts	–	p = 0,808

4.3.4. Antibakteriālā jutība

Pārbaudītie *S. aureus* izolāti bija jutīgi pret cefoksitīnu, ceftriaksonu, ampicilīnu/sulbaktāmu, amoksicilīnu/klavulānskābi, norfloksacīnu, amikacīnu, klindamicīnu, hloramfenikolu, bet rezistenti pret benzilpenicilīnu un ampicilīnu 75,6 % un eritromicīnu 14,6 % gadījumu (sk. 4.9. tabulu). No izolētajiem *S. aureus* izolātiem 1 bija MRSA, jo bija rezistents pret cefoksitīnu.

4.9. tabula

Antibakteriālā rezistence starp *S. aureus* izolātiem, kas izolēti no veseliem indivīdiem

		Antibakteriālā rezistence (%)										
Izolāts	**Izolāti (n)**	**FOX**	**CRO**	**BP**	**AMP**	**AMS**	**AUG**	**NOR**	**AK**	**E**	**CD**	**C**
S. aureus	41	2,4	2,4	75,6	75,6	2,4	2,4	2,4	2,4	14,6	0	4,9

FOX, cefoksitīns; CRO, ceftriaksons; BP, benzilpenicilīns; AMP, ampicilīns; AMS, ampicilīns/sulbaktāms; AUG, amoksicilīns/klavulānskābe; NOR, norfloksacīns; AK, amikacīns; E, eritromicīns; CD, klindamicīns; C, hloramfenikols.

Gramnegatīvās baktērijas bija jutīgas pret visām pārbaudītajām antibiotikām (sk. 4.10. tabulu). Tikai 1 *Acinetobacter junii* izolāts bija rezistents pret amikacīnu. Neviens no izolētajiem *K. pneumoniae* variantiem nebija paplašināta spektra beta-laktamāzes (*ESBL*) ražotājs. Netika konstatētas statistiski nozīmīgas korelācijas starp antibakteriālo jutību un biofilmas veidošanas spēju.

4.10. tabula

Antibakteriālā rezistence starp *K. pneumoniae, P. aeruginosa, Serratia liquefaciens* un *Acinetobacter* spp., kas izolēti no veseliem indivīdiem

		Antibakteriālā rezistence (%)											
Izolāti	**Izolāti (n)**	**AUG**	**TZP**	**CTX**	**CAZ**	**ETP**	**IMP**	**MEM**	**CIP**	**GM**	**SXT**	**AK**	**LEV**
K. pneumoniae	7	0	0	0	0	0	0	0	0	0	0	–	–
P. aeruginosa	2	–	0	–	0	–	0	0	0	–	–	0	–
Acinetobacter spp.	5	–	–	–	–	–	0	–	0	0	0	20	0
S. liquefaciens	1	0	0	0	0	0	0	0	0	0	0	–	–

AUG, amoksicilīns/klavulānskābe; TZP, piperacilīns/tazobaktāms; CTX, cefotaksīms; CAZ, ceftazidīms; ETP, ertapenēms; IMP, imipenēms; MEM, meropenēms; CIP, ciprofloksacīns; GM, gentamicīns; SXT, trimetoprims/sulfametoksazols; AK, amikacīns; LEV, levofloksacīns.

Kopsavilkums. Veselu cilvēku aukslēju mandeles kolonizē pārsvarā grampozitīvie mikroorganismi, *S. aureus* 45 % gadījumu. Kolonizē arī gramnegatīvie mikroorganismi, piemēram, *K. pneumoniae* 7 %, *Acinetobacter* spp. 5,5 %. Šie ierosinātāji var būt oportūnistiskās infekcijas etioloģijā. Pierādīts, ka 95 % no identificētajiem *S. aureus* ir biofilmu veidojošie izolāti. Biofilmas ir veselu cilvēku audus kolonizējošo patogēno baktēriju dabiski eksistējošā forma. Gramnegatīvās baktērijas biofilmas veidoja ievērojami retāk. Visas izdalītās baktērijas no veselu cilvēku aukslēju mandelēm bija jutīgas pret antibiotikām.

4.4. *Staphylococcus aureus* kolonizācijas novērtējums pacientiem, kuriem veikta tonsilektomija recidivējoša tonsilīta dēļ

Pētījums ir aprakstīts Renātas Klagišas, Kārļa Rāceņa, Renāra Broka, Ligijas Ķīses un Jutas Kroičas rakstā *Evaluation of Staphylococcus aureus Colonization in Adult Patients Undergoing Tonsillectomy for Recurrent Tonsillitis*. *Pathogens.* 2022, 11, 427. https://doi.org/10.3390/pathogens11040427

Pētījumā tika iekļauti 16 pacienti, no kuriem 8 bija sievietes un 8 vīrieši vecumā no 21 līdz 50 gadiem ar vidējo vecumu 29 gadi (± 7,23). Rīgā dzīvoja 7 pacienti, pārējie – citās Latvijas pilsētās.

No aukslēju mandeļu kriptām visbiežāk izdalītā baktērija bija *S. aureus*, kas bija vienīgais izdalītais mikroorganisms 6 pacientiem un kopā ar normālo orālo mikrobiotu vai citiem potenciāli patogēniem mikroorganismiem 10 pacientiem. Tika izolēti 16 *S. aureus* izolāti un pārbaudītas biofilmas veidošanas spējas, un 15 no 16 bija biofilmas veidojoši izolāti. Turklāt 1 no izolātiem bija stingras biofilmas veidotājs, 5 no16 izolātiem bija mērenas un 9 no 16 bija vājas biofilmas veidotājs, un 1 *S. aureus* izolāts biofilmu neveidoja (sk. 4.11. tabulu).

Gadu pēc tonsilektomijas 8 *S. aureus* izolāti tika izdalīti 6 no 16 pacientiem – kultūrās no žāvas 3 no 16 pacientiem, no deguna priekštelpas 4 no 16 pacientiem un no padušu paraugiem 1 no 16 pacientiem. No žāvas paraugiem 1 no 3 bija stingru biofilmu ražotājs un 2 no 3 *S. aureus* izolāti bija vājas biofilmas ražotāji. No deguna priekštelpas paraugiem 1 no 4 bija mērenas biofilmas ražotājs, 1 no 4 bija vājas biofilmas ražotājs un 2 no 4 *S. aureus* izolāti biofilmu neveidoja. No padušu paraugiem 1 *S. aureus* izolāts biofilmu neveidoja. Tikai 1 pacientam bija *S. aureus* žāvas, deguna un padušu paraugos, un tas bija vājas biofilmas ražotājs (sk. 4.11. tabulu).

S. aureus tika izolēts tikai tonsilektomijas laikā 10 pacientiem. Šajos gadījumos *S. aureus* bija RT izraisītājs. Pēc tonsilektomijas *S. aureus* bija žāvā vai deguna priekštelpā, bet *S. aureus* celmiem tika konstatētas dažādas biofilmas veidošanās pakāpes 4 pacientiem. Šajos gadījumos pacientiem bija nosliece uz *S. aureus* kolonizāciju. *S. aureus* ar vienādu biofilmas ražošanas spēju tika izolēts no aukslēju mandelēm tonsilektomijas laikā un 1 gadu pēc tonsilektomijas no žāvas, deguna priekštelpas un padusēm 2 pacientiem. Šajos gadījumos *S. aureus* bija daļa no pacientu mikrobiotas (sk. 4.11. tabulu).

4.11. tabula

Pacientu raksturojums un mikrobioloģisko izmeklējumu rezultāti tonsilektomijas laikā un gadu pēc tās

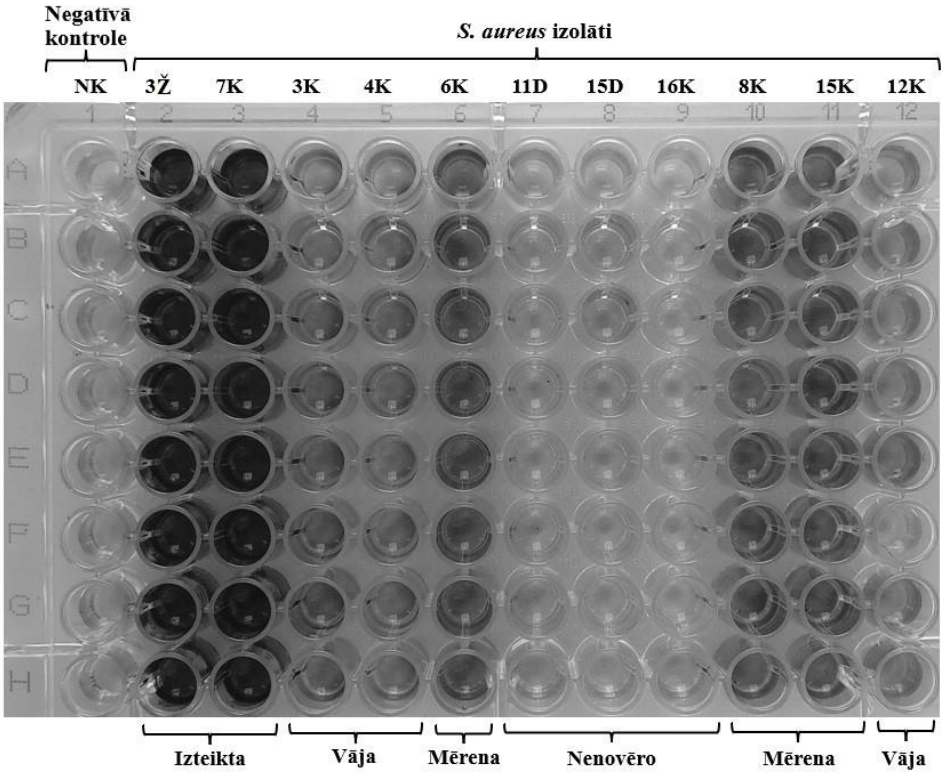
Nr.	**Mikroorganismi**	***S. aureus* biofilmas**				**Interpretācija**
		TE laikā	**Pēc TE**			
		Kriptas	**Žāva**	**Deguna priekštelpa**	**Paduses**	
1	*S. aureus* + orālā mikrobiota	1K vāja	0	0	0	Izraisītājs
2	*S. aureus*	2K vāja	0	2D mērena	0	Nosliece uz kolonizāciju
3	*S. aureus* + *Candida* spp.	3K vāja	3Ž stingra	0	0	Nosliece uz kolonizāciju
4	*S. aureus* + orālā mikrobiota	4K vāja	4Ž vāja	0	0	Normālā orālā mikrobiota
5	*S. aureus* + orālā mikrobiota	5K vāja	5Ž vāja	5D vāja	5P vāja	Normālā orālā mikrobiota
6	*S. aureus* + *S. epidermidis*	6K mērena	0	0	0	Izraisītājs
7	*S. aureus* + orālā mikrobiota	7K stingra	0	0	0	Izraisītājs
8	*S. aureus*	8K mērena	0	0	0	Izraisītājs
9	*S. aureus* + *S. pneumoniae* + orālā mikrobiota	9K vāja	0	0	0	Izraisītājs
10	*S. aureus* + *K. pneumoniae* + *Candida* spp. + orālā mikrobiota	10K vāja	0	0	0	Izraisītājs
11	*S. aureus*	11K mērena	0	11D biofilmas nav	0	Nosliece uz kolonizāciju

4.11. tabulas turpinājums

Nr.	Mikroorganismi	*S. aureus* biofilmas				Interpretācija
		TE laikā	Pēc TE			
		Kriptas	Žāva	Deguna priekštelpa	Paduses	
12	*S. aureus*	12K vāja	0	0	0	Izraisītājs
13	*S. aureus* + *N. subflava* + *H. influenzae* + *S. anginosus* + *P. intermedia* + orālā mikrobiota	13K vāja	0	0	0	Izraisītājs
14	*S. aureus*	14K mērena	0	0	0	Izraisītājs
15	*S. aureus* + orālā mikrobiota + *S. agalactiae*	15K mērena	0	15D biofilmas nav	0	**Nosliece uz kolonizāciju**
16	*S. aureus*	16K biofilmas nav	0	0	0	**Izraisītājs**

TE, tonsilektomija; K, kriptas; Ž, žāva; D, deguna dobums; P, paduses.

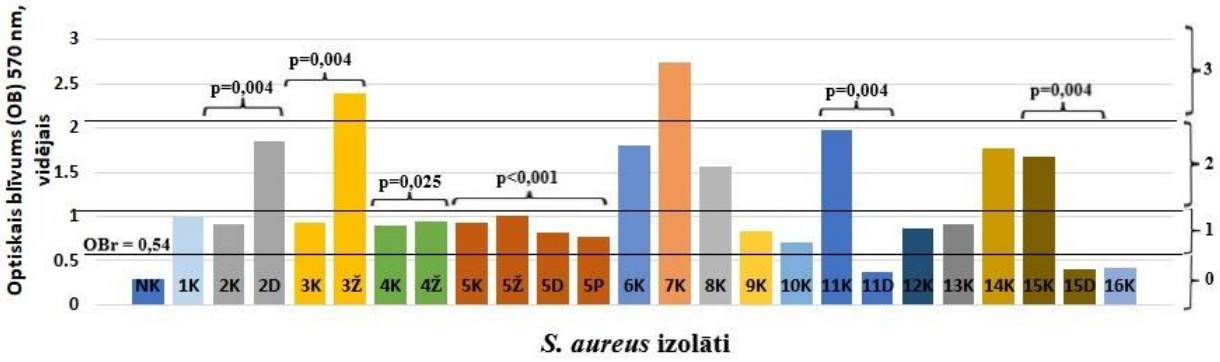
4.5. attēlā parādīta 11 *S. aureus* izolātu biofilmas veidošanās 96 bedrīšu mikroplatēs. Kristālvioletā absorbcija ir proporcionāla adhēzijas šūnām un biofilmas koncentrācijai.



4.5. attēls. ***S. aureus* izolātu biofilmas veidošana 96 bedrīšu mikroplatē**

Platē 11 izolāti un negatīvā kontrole (sterils buljons) ar 8 iedobēm katram izolātam. Krāsošana veikta ar kristālvioleto, kas atšķir izteiktu (3Ž, 7K), mērenu (6K, 8K, 15K), vāju (3K, 4K, 12K) biofilmas veidošanas pakāpi un biofilmas veidošanos nenovēro (11D, 15D, 16K).

Kristālvioletā krāsa, kas pievienota šūnām, kuras veido biofilmas uz mikroplatēm, tika mērīta kvantitatīvi. Baktēriju biofilmas optiskais blīvums (OB) tika mērīts pie 570 nm viļņa garuma ar mikroplates spektrofotometru. Katra izolāta OB vērtība bija izteikts kā skaitlis. Visas izdalīto izolātu biofilmu vidējās OB vērtības ir apkopotas 4.6. attēlā.



4.6. attēls. ***S. aureus* 24 izolātu biofilmas veidošanas spēja uz mikroplatēm**

Stabiņi attēlo OB vidējās vērtības (mērītas pie viļņa garuma 570 nm). TSB ar 1 % glikozi kā negatīvā kontrole (NK). Robežvērtība (OBr) un biofilmu veidošanas pakāpes ir atzīmētas ar horizontālām līnijām: 0 – biofilmas neražotāji; 1 – vāji biofilmas ražotāji; 2 – mērenas biofilmas ražotāji; 3 – stingras biofilmas ražotāji. Atšķirības starp *S. aureus* izolātu biofilmu veidošanās spējām bija analizētas, izmantojot *Mann-Whitney* U testu 2 izolātu analīzei un *Kruskal-Wallis* testu 4 izolātu analīzei, un ir atzīmētas p vērtības. *S. aureus* izolāta kods – numurs apzīmē pacientu un burts – parauga iegūšanas vietu (K – kriptas; Ž – rīkle; D – deguna priekštelpa; P – paduses).

Pret benzilpenicilīnu un ampicilīnu bija rezistenti 14 izolāti. Kā MRSA tika identificēts 1 izolāts, jo bija rezistents pret cefoksitīnu. Visi izolāti bija jutīgi pret ciprofloksacīnu paaugstinātā koncentrācijā, savukārt 1 izolāts bija arī jutīgs pret klindamicīnu paaugstinātā koncentrācijā (sk. 4.12. tabulu).

4.12. tabula

Antibiotiku rezistence starp *S. aureus*, kas izolēti no pacientiem ar RT

		Antibiotikas										
		FOX	**CRO**	**BP**	**AMP**	**AMS**	**AUG**	**CIP**	**AK**	**E**	**CD**	**C**
***S. aureus* izolāti**	**1K**	J	J	R	R	J	J	I	J	J	J	J
	2K	J	J	J	J	J	J	I	J	J	J	J
	2D	J	J	R	R	J	J	I	J	J	J	J
	3K	J	J	R	R	J	J	I	J	J	J	J
	3Ž	J	J	J	J	J	J	I	J	J	J	J
	4K	R	R	R	R	R	R	I	J	J	J	J
	4Ž	J	J	R	R	J	J	I	J	J	J	J
	5K	J	J	R	R	J	J	I	J	J	J	J
	5Ž	J	J	J	J	J	J	I	J	J	J	J
	5D	J	J	J	J	J	J	I	J	J	J	J
	5P	J	J	J	J	J	J	I	J	J	J	J
	6K	J	J	R	R	J	J	I	J	J	J	J
	7K	J	J	R	R	J	J	I	J	J	J	J
	8K	J	J	R	R	J	J	I	J	J	J	J
	9K	J	J	R	R	J	J	I	J	J	J	J
	10K	J	J	R	R	J	J	I	J	J	J	J
	11K	J	J	J	J	J	J	I	J	J	J	J
	11D	J	J	J	J	J	J	I	J	J	J	J
	12K	J	J	R	R	J	J	I	J	J	J	J
	13K	J	J	R	R	J	J	I	J	J	I	J
	14K	J	J	J	J	J	J	I	J	J	J	J
	15K	J	J	R	R	J	J	I	J	J	J	J
	15D	J	J	R	R	J	J	I	J	J	J	J
	16K	J	J	J	J	J	J	I	J	J	J	J

FOX, cefoksitīns; CRO, ceftriaksons; BP, benzilpenicilīns; AMP, ampicilīns; AMS, ampicilīns/sulbaktāms; AUG, amoksicilīns/klavulānskābe; CIP, ciprofloksacīns; AK, amikacīns; E, eritromicīns; CD, klindamicīns; C, hloramfenikols. J, jutīgs; R, rezistents; I, jutīgs paaugstinātā koncentrācijā.

Kopsavilkums. No aukslēju mandeļu kriptām izdalītie *S. aureus* bija ar augstāku biofilmas veidošanas kapacitāti salīdzinājumā ar izolātiem no citām ķermeņa vietām. Gadu pēc tonsilektomijas *S. aureus* netika izdalīts 10 no 16 pacientiem. Tonsilektomija ievērojami samazina *S. aureus* nēsāšanu un ir efektīva RT ārstēšanas metode.

5. Diskusija

Pasaulē plaši tiek pētīti RT patoģenēzes mehānismi, tiek veikti pētījumi gan mikrobioloģijas, gan imunoloģijas jomā, meklējot objektīvus biomarķierus, lai identificētu pacientus ar RT. Pašreizējās RT diagnostikas metodes balstās tikai uz klīnisko ainu un mikrobioloģiskiem izmeklējumiem, kuri neatspoguļo situāciju mandeļaudos. Šobrīd galvenais izšķirošais faktors, izskatot indikācijas tonsilektomijai, tiek izmantots tonsilītu biežums iepriekšējo trīs gadu laikā (*Paradise* kritēriji). Objektīvu biomarķieru neesamības dēļ grūtības ir klīniskajā praksē, piemēram, paiet 1–3 gadi, līdz noteikta RT diagnoze un lemts par tonsilektomijas nepieciešamību, kā arī zinātnē RT pacientu pētījumā iekļaušanas kritēriji balstās anamnēzes datos, ierakstos medicīniskajā dokumentācijā.

Veicot literatūras analīzi, secinājām, ka ir vairāki pētījumi, kuros salīdzināti RT pacientu un veselo indivīdu dati, bet nozīmīgas atšķirības netika atrastas, un veikti plaši mikrobioloģiski PTA pētījumi, bet bez salīdzinošās grupas. Promocijas darbā tādēļ tika pētītas trīs grupas: RT, PTA pacienti un veselie indivīdi.

RT patoģenēze nav pilnībā skaidra, tomēr zināms, ka tajā iesaistītas baktērijas ar antibiotiķu tolerances mehānismiem. Mūsu darbā ar punktēšanas biopsijas adatu tika iegūti RT un PTA pacientu mandeļaudu paraugi, izvairoties no normālās orālās mikrobiotas, un veikta to bakterioloģiska testēšana objektīvu marķieru atrašanai.

Izaicinoša ir bakterioloģiskā izmeklēšana no vietām ar augstu kolonizāciju, pozitīvs kultivēšanas rezultāts bija 60 (60,6 %) RT pacientiem, 24 (82,8 %) PTA pacientiem un 54 (59,3 %) veseliem indivīdiem. Pētāmajās grupās bija plaša mikroorganismu daudzveidība, dominēja grampozitīvi mikroorganismi un visbiežāk izdalītā patogēnā baktērija bija *S. aureus*, vienīgi PTA grupai konstatēja augstu *Candida* spp. izdalīšanas biežumu. *S. aureus* izolātiem plaša antibakteriālā rezistence netika konstatēta nevienā no pētāmajām grupām, bet izteiktas biofilmu veidošanas spēja novēroja *S. aureus* izolātiem no veselo indivīdu mandeļaudiem. Jādomā, ka gļotādu biofilmas ir dabiskas baktēriju pastāvēšanas forma un neliecina par tonsilopātiju atšķirībā no pētījumiem par biofilmām, kas saistītas ar medicīniskajām protēzēm vai svešķermeņiem.

Antibakteriālā testēšana veikta atbilstoši *EUCAST* standartiem, izolāti testēti uz daudziem antibiotiķiem, lai raksturotu izolātu antibakteriālo rezistenci, neskatoties uz to, ka klīniskajā praksē RT terapijā tādus antibiotiķus nelieto, piemēram, *S. aureus* gadījumā cefoksitīns, lai raksturotu rezistenci pret meticilīnu, un gramnegatīvu baktēriju gadījumā meropenēms, lai lemtu par turpmāko testēšanu uz karbapenemāzēm.

5.1. Mikroorganismu kolonizācijas, biofilmu veidošanas un antibakteriālās jutības analīze recidivējoša tonsilīta un paratonsilāra abscesa pacientiem

RT un PTA ir slimības ar dažādu klīnisko ainu, slimības gaitu un prognozēm. Abām slimībām ir kopīgās iezīmes, pirmkārt, tās ir bieži sastopamas otorinolaringologa praksē, otrkārt, to etioloģiskais faktors visbiežāk ir baktērijas, un, treškārt, antibakteriālās terapijas neefektivitātes gadījumā abas slimības var veiksmīgi ārstēt ķirurģiski. Veiksmīgas ārstēšanas plāns abām infekciju slimībām var būt saistīts ar līdzīgu etioloģiju (Zautner et al., 2010).

Streptococcus pyogenes (S. pyogenes) ir biežākais akūta bakteriāla tonsilīta ierosinātājs imūnkompetentiem pieaugušajiem. Ir pieņemts, ka ir akūta tonsilīta viens etioloģiskais faktors, bet RT etioloģija ir multifaktoriāla divu un vairāku baktēriju sugu klātbūtne (Babaiwa, 2013; Katkowska et al., 2017). Mūsu pētījumā tika novērots, ka pacientiem ar RT bija liela mikroorganismu dažādība, dominēja polikultūras. Šāda atrade ir novērota arī citām slimībām, piemēram, gingivīta slimību gadījumos (Jorgensen et al., 2015).

Mūsu pētījumā *Streptococcus* spp. izdalīšanas biežums bija zems, RT gadījumā tika izdalīts viens streptokoku izolāts, bet PTA gadījumā – trīs streptokoku izolāti. *S. pyogenes* izolāti bija izdalīti kopā ar citiem mikroorganismiem četriem pacientiem ar RT, bet netika izdalīti nevienam pacientam ar PTA. Arī citos pētījumos RT pacientiem ir konstatēts zems streptokoku (1,7–5 %) izolācijas biežums, ir pierādīts, ka uz aukslēju mandeļu virsmas tie satopami biežāk nekā mandeļaudos (Syryło et al., 2007; Alasil et al., 2013). RT patoģenēzē *S. pyogenes* loma ir pārvērtēta vai samazinājusies gadu gaitā (Lindroos, 2000; Zautner et al., 2010; Kostić et al., 2022). RT un PTA pacientiem ātro streptokoku testu izmantošana un antistreptolizīna O (ASO) noteikšana nav lietderīga sakarā ar zemu *S. pyogenes* īpatsvaru. Mikroorganismu izdalīšanas biežums atkarīgs no parauga iegūšanas veida. *Zautner* et al. (2010) pētījumā tika analizētas aukslēju mandeļu šūnu suspensijas un konstatēts, ka *S. aureus* prevalē pacientiem ar RT (57,7 %), bet *S. pyogenes* – pacientiem ar PTA (20,2 %) (Zautner et al., 2010). *Vaikjärv* et al. (2016) pētījumā tika atspoguļots, ka PTA abscesa gadījumā aukslēju mandeļu nišu biopsijas ir labāks materiāls mikrobioloģiskai analīzei nekā PTA strutu materiāls, jo vienā materiālā tie atklāj vairāk baktēriju (Vaikjärv et al., 2016). *Streptococcus* spp. bija biežākais patogēns gan aukslēju mandeļu nišu biopsiju, gan strutu paraugos, bet *Staphylococcus* spp. bija biežākais patogēns aukslēju mandeļu nišu biopsiju paraugos, strutu paraugos stafilokokus nekonstatēja vispār (Vaikjärv et al., 2016). Mūsu pētījumā izvēlējāmies aukslēju mandeļu kriptu biopsijas, lai iegūtais materiāls būtu informatīvs un RT un PTA grupas būtu savstarpēji salīdzināmas.

Mūsu pētījumā *Streptococcus anginosus* (*S. anginosus*) bija atklāts tikai RT pacientiem, bet nevienam PTA pacientam. Cits pētījums pierādīja, ka *S. anginosus* grupas baktērijas sastopamas biežāk pacientiem ar PTA atkārtošanos nekā pacientiem ar PTA izārstēšanos. Pētījuma autori *S. anginosus* grupas baktērijas klātbūtni saista ar PTA atjaunošanos (Wikstén et al., 2017).

Mūsu pētījumā tika konstatēts augsts *Candida* spp. biežums. RT pacientiem *Candida* spp. bija izolētas 8,08 % (n = 8) gadījumu, bet PTA pacientiem 48,23 % (n = 14) gadījumu. Citos pētījumos bija konstatēts zemāks *Candida* spp. izdalīšanas biežums. *Katkowska* et al. (2017) pētījumā konstatēja *Candida* spp. RT pacientu mandeļaudu paraugos 2,5 % gadījumu, aukslēju mandeļu virsmas paraugos 8,3 % gadījumu, iztriepē no žāvas pirms tonsilektomijas 9,3 % gadījumu (Katkowska et al., 2017). *Zautner* et al. (2010) savā pētījumā ziņoja, ka RT pacientu mandeļaudos *Candida* spp. sastopama 12,8 % gadījumu, bet PTA pacientiem – 4,9 % gadījumu (Zautner et al., 2010). *Slouka* et al. (2020) ziņoja, ka PTA strutu aspirātos 2,3 % gadījumu konstatētas *Candida* spp. (Slouka et al., 2020). Mūsu pētījuma rezultāti līdzinās rezultātiem, ko *Jokinen* et al. (1976) aprakstījis savā pētījumā, kur 147 pacientiem ar hronisku tonsilītu *Candida albicans* (*C. albicans*) konstatēta 41,4 % gadījumu, bet veseliem indivīdiem *C. albicans* izdalīšanas biežums bija 51,5 % (Jokinen et al., 1976). Sakarā ar tik augstu izdalīšanas biežumu tika novērtēta arī sēnīšu patoģenitāte, izmantojot paraugu histoloģisku izmeklēšanu. Histoloģiskā izvērtēšanā netika atklātas sēnīšu patoģenitātes pazīmes, jo sēnītes bija atrodamas tikai aukslēju mandeļu kriptās, bez granulomatoza iekaisuma ap tiem (Jokinen et al., 1976). Citā pētījumā novērtēts aukslēju mandeļu mikobioms starp indivīdiem ar cilvēka imūndeficīta vīrusu (HIV) un indivīdiem bez tā (Fukui et al., 2018). Aukslēju mandeļu mikobioms būtiski neatšķiras starp indivīdiem ar HIV un indivīdiem bez HIV infekcijas (Fukui et al., 2018). Balstoties uz mūsu pētījuma rezultātiem, varam secināt, ka *Candida* spp. varētu būt mikrobioloģiskais indikators PTA grupā. *Candida* spp. var būt procesa hroniskas norises indikators, kad, pirmkārt, jāatrisina pamatproblēma, kas var būt bakteriāla vai strukturāla, un, otrkārt, būtu apsverama antifungāla terapija tikai tad, ja pamatproblēma nepadodas antibakteriālai terapijai.

Šajā pētījumā gramnegatīvi mikroorganismi neieņēma nozīmīgu lomu. *K. pneumoniae* ir pazīstama kā stingru biofilmu veidotāja (Wang et al., 2020), bet mūsu pētījumā *K. pneumoniae* bija ne tikai reti konstatēta, bet uzrādīja arī zemas biofilmu veidošanas spējas. Neviens no *K. pneumoniae* izdalītajiem celmiem nebija stingru biofilmu veidotājs. Ir zināms, ka *Haemophilus influenzae* (*H. influenzae*) var tikt izdalīta no mandeļaudiem, bet mūsu pētījumā *H. influenzae* tika izdalīta tikai divos gadījumos RT pacientu grupā. Netika konstatēta

statistiski nozīmīga saistība starp gramnegatīvu mikroorganismu un biofilmu producējoša celma klātbūtni mandeļaudos nedz pacientiem ar RT, nedz pacientiem ar PTA.

Pacientiem ar PTA A grupas *Streptococcus* spp. bija biežāk izdalīts ziemā un pavasarī nekā vasarā (Klug, 2017). Sezonālas atšķirības mūsu pētījumā netika konstatētas. Tādi iekaisuma rādītāji kā leikocīti, CRP bija statistiski nozīmīgi paaugstināti PTA pacientiem, salīdzinot ar RT pacientiem. PTA pacienti tika iekļauti pētījumā akūta iekaisuma stadijā, tas izskaidro augstos iekaisuma rādītājus.

S. aureus izolāti uzrādīja rezistenci pret benzilpenicilīnu un ampicilīnu, kas ir novērots arī citos pētījumos. Piemēram, *Katkowska* et al. (2017) pētījumā RT pacientu *S. aureus* izolāti bija rezistenti pret penicilīnu 79 % gadījumu un pret ampicilīnu 63,2 % gadījumu, bet viens *S. aureus* izolāts bija MRSA (Katkowska et al., 2017). Pētījumā nenovērojām sakarību starp antibakteriālo rezistenci un biofilmu veidošanās intensitāti. Lai gan vairākos pētījumos apgalvots, ka intensīva biofilmu veidošanās ir saistāma ar izteiktāku antibakteriālo rezistenci, *Ma* et al. (2019) savā pētījumā demonstrēja, ka palielināta biofilmu veidošanās rezultējas ar pretēju efektu, antibakteriālā tolerance samazinās (Ma et al., 2019). Mūsu pētījuma dati neapstiprina hipotēzi, ka PTA pacientu uzsējumos dominē biofilmu veidojošie izolāti. Tieši otrādi, PTA pacientu uzsējumos no septiņiem *S. aureus* izolātiem divi bija vāju biofilmu veidotāji, bet pieci bija biofilmu neveidojoši izolāti. Šie rezultāti liecina, ka izdalītie *S. aureus* varētu būt savvaļas tipa izolāti, nevis endogēnas infekcijas ierosinātāji.

Biofilmas tiek raksturotas kā baktēriju kopiena, ko apņem pašu ražotā ārpussūnu polimēru vielas matrica (Forson et al., 2020). Tiek veikti pētījumi, lai izprastu mijiedarbību starp mikroorganismiem biofilmās. Mijiedarbība starp *C. albicans* un *Staphylococcus* spp. acīmredzot ir savstarpēji sinerģiska, un par to tiek ziņots arvien vairāk (Schnurr et al., 2021). Pētījumu dati liecina, ka sēnīšu šūnas spēj modulēt antibiotiķu darbību un baktērijas var ietekmēt antifungālo aktivitāti jauktās sēnīšu un baktēriju biofilmās (Adam et al., 2002). Prostaglandīns E2, ko izdala *C. albicans*, veicina *S. aureus* augšanu jauktās biofilmās (Krause et al., 2015). Aukslēju mandeļu hronisks iekaisums var tikt skaidrots ar baktēriju kopienas aizsargājošo mijiedarbību un lielu mikroorganismu daudzveidību.

5.2. Uz aukslēju mandeļu virsmas un aukslēju mandeļu kriptās esošo patogēno mikroorganismu novērtējums pacientiem ar recidivējošu tonsilītu

Mikroorganismu sastopamības biežums uz aukslēju mandeļu virsmas, aukslēju mandeļu kriptās un mandeļaudos, iegūto kultūru rezultātu precizitāte ir analizēta daudzos pētījumos (Brook et al., 1980; Lindroos, 2000; Khadilkar & Ankle, 2016; Sarkar et al., 2017; Dickinson et al., 2020). Precīza tonsilīta ierosinātāja identifikācija ļauj uzlabot antibakteriālās terapijas

izvēli, un iespējams izvairīties no plānveida tonsilektomijas (Sarkar et al., 2017). Pētījumā liela uzmanība tika veltīta *S. aureus* analīzei, jo tas bija visbiežāk izdalītais patogēns. Arī citos pētījumos pierādīts, ka *S. aureus* ir būtiska loma RT patoģenēzē, jo *S. aureus* spēj uzturēties gan gļotādas biofilmās, gan pastāvēt intracelulāri (Zautner et al., 2010; Zautner, 2012; Stępińska et al., 2014; Cavalcanti et al., 2019). *K. pneumoniae* noturīgu biofilmu veidošanas spējas ir apstiprinātas pētījumos. Pateicoties *K. pneumoniae* biofilmām, tās spēj kolonizēt elpceļus, aizdeguni, mandeles (Alasil et al., 2013; Wang et al., 2020). *Sarkar* et al. (2017) savā pētījumā konstatēja, ka *S. aureus*, A grupas β-hemolītiskie streptokoki un *Klebsiella* spp. ir biežākie izolāti paraugos gan no aukslēju mandeļu virsmas, gan mandeļaudu biopsijas, ar augstāku minēto mikroorganismu klātbūtni mandeļaudos (Sarkar et al., 2017). Mūsu pētījumā tika pierādīts, ka *S. aureus* bija biežāk sastopams paraugos, kas iegūti no aukslēju mandeļu kriptām ar birstīti, bet *K. pneumoniae* – mandeļaudos, kas iegūti ar punktēšanas biopsijas adatu.

5.3. No veselu indivīdu aukslēju mandeļu kriptām iegūto patogēno mikroorganismu biofilmu veidošanas, antibakteriālās jutības novērtējums

Mutes dobumā un rīklē ir daudzas nišas baktēriju kolonizācijai. Tā kā aukslēju mandeļu infekcija, visticamāk, rodas no baktērijām, kas atrodas aukslēju mandeļu kriptās vai parenhīmā, nevis no tām, kas atrodas uz virsmas, mēs pētījām aukslēju mandeļu kriptu mikrobiotu kā visnozīmīgāko cēloni tonsilopātijas attīstībā (Khadilkar & Ankle, 2016; de Martin et al., 2021). Veselo indivīdu pētījumā mēs izolējām un analizējām nozīmīgus rīkles un elpceļu patogēnus – *S. aureus*, *K. pneumoniae*, *Pseudomonas aeruginosa* (*P. aeruginosa*) un *Acinetobacter* spp. (Hamilos, 2019; Zaatout, 2021).

Staphylococcus spp. primārā ekoloģiskā niša ir nāsis, tomēr mutes dobums ir nozīmīgs šo baktēriju rezervuārs, un dažiem pieaugušajiem pacientiem novēro mutes dobuma kolonizāciju (Kearney et al., 2020). *Albrich & Harbarth* (2008) pētījumā organisma citu rajonu, izņemot degunu (angliski *extranasal*), kolonizācijas vietas bija saistītas ar pastāvīgu *S. aureus* nēsāšanu (Albrich & Harbarth, 2008). Pētījumā, ko Amerikas Savienotās Valstīs veica *Hansons* et al. (2018), tika ziņots, ka 6,2 % pieaugušo *S. aureus* nēsāja tikai deguna priekštelpā, 18,6 % tikai rīkles mutes daļā (latīniski *oropharynx*) un 19,8 % abās vietās (Hanson et al., 2018). Ir ziņots, ka studentu populācijās *S. aureus* mutes dobumā nēsātāju skaits sasniedz no 17 līdz 48 procentiem (Smith et al., 2003; Blomqvist et al., 2015). Veseliem Zviedrijas zobārstniecības studentiem *S. aureus* izplatība bija 44,6 %; starp tiem MRSA netika atklāts (Blomqvist et al., 2015). Konstatēts, ka veselības aprūpes darbiniekiem MRSA ir 23,7 % (Albrich & Harbarth, 2008). Citu autoru pētījumos minēts, ka MRSA izplatība veseliem nēsātājiem svārstās no 1,5 līdz 26 % (Petti & Polimeni, 2011; Roberts et al., 2011; Laheij et al., 2012). Mūsu pētījumā

iesaistītiem veseliem indivīdiem *S. aureus* bija visizplatītākais izolētais patogēns; tas tika izolēts 45 % gadījumu, un MRSA tika izolēts 1,1 % gadījumu, kas atbilst iepriekš veikto pētījumu rezultātiem.

Jeong et al. (2007) pētījumā *K. pneumoniae* tika izolēts no RT pacientu aukslēju mandeļu serdes paraugiem 6,7 % gadījumu, bet no aukslēju mandeļu hipertrofijas pacientu – 1,5 % gadījumu (Jeong et al., 2007). Mūsu iegūtie dati parādīja, ka *K. pneumoniae* bija sastopams arī veselu cilvēku aukslēju mandeļu kriptās 7,7 % gadījumu.

Vairākos pētījumos ir analizēta ekstracelulārā vai intracelulārā *P. aeruginosa* loma periodontālu vai plaušu slimību izcelsmē (Mirzaei et al., 2020; Li et al., 2021). Citi pētījumi ir ziņojuši par 1,4–3,8 % *P. aeruginosa* izplatību RT pacientu aukslēju mandeļu paraugos (Loganathan et al., 2006; Jeong et al., 2007; Al Ahmary et al., 2012) un 0,9 % izplatību aukslēju mandeļu paraugos pacientiem ar aukslēju mandeļu hipertrofiju (Jeong et al., 2007). Mūsu pētījums parādīja, ka *P. aeruginosa* bija sastopama 2,2 % gadījumu veselu indivīdu aukslēju mandeļu kriptās. Starp *Acinetobacter* izolātiem *Acinetobacter baumannii* netika atklāts. Patogēna izolācijas no aukslēju mandeļu paraugiem daudzveidība un izplatība var atšķirties atkarībā no paraugu ņemšanas metodes, piemēram, mandeļu virsmas uztriepes var būt mazāk informatīvas nekā mandeļu kriptu materiāls (Klagisa et al., 2021b).

Aukslēju mandeļu kriptas ir piemērota vieta biofilmas veidošanai. Aukslēju mandeļu kriptas spēj uzkrāt detrītu, un tā mineralizācija izraisa tonsilolītu veidošanos (Ferguson et al., 2014). Tonsilolītiem raksturīgas dinamiskas biofilmas, kas līdzīgas dentālām biofilmām (Stoodley et al., 2009). Mūsu pētījums parādīja, ka veseliem cilvēkiem 61 % *S. aureus* izolātu un 40 % gramnegatīvo baktēriju izolātu piemita mērenas vai izteiktas biofilmas veidošanas spējas. Pētījums apstiprināja, ka biofilmas veidošanās ir daļa no normāla baktēriju dzīvesveida un ka biofilmas var pastāvēt veselu indivīdu aukslēju mandelēs. *Penesyan* et al. (2021) pētījumā biofilma tika aprakstīta kā galvenais mikrobu dzīvesveids; biofilmas veic svarīgu funkciju, mikrobiem nodrošinot aizsargājošu vidi, kurā tiek ģenerēta genotipiskā un fenotipiskā daudzveidība (Penesyan et al., 2021). Slimiem un veseliem indivīdiem biofilmas īpašības var atšķirties. *Chervinets* et al. (2021) ziņoja, ka periodontīta pacientu mutes dobuma mikrobiotai bija lielāka spēja pieķerties gļotādas šūnām nekā veseliem cilvēkiem, kā arī novēroja pastiprinātu spēju veidot biofilmas un uzrādīt patogēnās īpašības (Chervinets et al., 2021).

Etioloģiskā aģenta lokalizācija biofilmās var veicināt rezistenci pret antibiotikām. Antibiotiku rezistence ir *S. aureus* galvenā problēma, īpaši MRSA. Ir ziņots par pieaugošu MRSA izplatību veseliem nēsātājiem, zobārstniecības studentu deguna paraugos sastopams līdz 21 % (Roberts et al., 2011). Mazāk zināma ir MRSA izplatība mutes dobumā. Tika pārbaudītas subgingivālās vietas un mēles virsma, un MRSA netika atklāta (Koukos et al., 2015). Mūsu

pētījums aukslēju mandeļu paraugos atklāja vienu (1,1 %) MRSA izolātu. Veseliem indivīdiem bija augsts *S. aureus* izolātu skaits ar rezistenci pret benzilpenicilīnu un ampicilīnu, tomēr neviens izolāts nebija rezistents pret klindamicīnu. Dati, kas iegūti par *S. aureus* antibakteriālo rezistenci, atbilst *Katkowska* et al. (2017) pētījuma rezultātiem (Katkowska et al., 2017). Klindamicīnu plaši izmanto zobārstniecībā, un daudzas klīnikas ir aizstājušas ar to penicilīnus (oksacilīnu un meticilīnu); klindamicīns tiek noteikts arī alerģijas pret beta-laktāmiem gadījumā (Blomqvist et al., 2015).

Ir izvirzīta hipotēze, ka infekciozajiem izolātiem ir atšķirīgs virulences arsenāls nekā tiem izolātiem, kas kolonizē veselus indivīdus (Blomqvist et al., 2015). Tomēr pētījumi nav pierādījuši, piemēram, ka *S. aureus* izolāti, kas izdalīti no infekcijas skarta mutes dobuma un infekcijām neskarta mutes dobuma, pārstāv dažādas fenotipisko un genotipisko īpašību apakšgrupas (Blomqvist et al., 2015). Tāpēc ir secināts, ka klasiskās oportūnistiskās infekcijas attīstās saimnieka un mikroorganisma nesabalansētas mijiedarbības rezultātā un ka infekcijas slimība turpinās tik ilgi, kamēr pastāv imūnkompromitēts stāvoklis (Blomqvist et al., 2015).

Vēlētos izcelt dažas šī pētījuma stiprās puses. Mēs izmantojām birstītes kā alternatīvu, neinvazīvu metodi aukslēju mandeļu paraugu savākšanai, izvairoties no nepieciešamības izmantot traumatisku metodi, lai iegūtu mandeļaudu paraugus. Tāpēc pētījumā varējām iekļaut veselus indivīdus bez aukslēju mandeļu slimību pazīmēm.

5.4. *Staphylococcus aureus* kolonizācijas novērtējums pieaugušajiem pacientiem, kuriem tiek veikta tonsilektomija recidivējoša tonsilīta dēļ

S. aureus kolonizācijas dominējoša ekoloģiska niša ir deguna priekštelpa, citas ķermeņa vietas, kas var tikt kolonizētas, ir paduses, cirkšņi, rīkle (Peacock et al., 2001). Samazinot *S. aureus* nēsāšanu deguna priekštelpā, izmantojot topiskos antibakteriālos līdzekļus, samazinās *S. aureus* nēsāšana arī citās vietās (Reagan, 1991). *S. aureus* viegli spēj atkārtoti kolonizēt deguna priekštelpu, rīkli un citas ķermeņa vietas dažus mēnešus pēc antibakteriālās terapijas (Mody et al., 2003; Coates et al., 2009). *S. aureus* nēsātājiem infekciju biežums ir augstāks nekā pacientiem, kuri nav *S. aureus* nēsātāji. Nēsātāji parasti ir inficēti ar to pašu celmu, ar ko bija kolonizēti (Lister & Horswill, 2014). Mūsu pētījumā vienu gadu pēc tonsilektomijas RT pacientiem tika novērtēta *S. aureus* klātbūtne rīklē, padusēs, deguna priekštelpā.

S. aureus nēsāšanu ietekmē gan bakteriāli, gan saimniekorganisma faktori. Stafilokoku infekcijas galvenie riska faktori ir vecums, blakusslimības vai imūndeficīts, ģenētiskie faktori, tiešs kontakts ar veselības aprūpes iestādi vai hospitalizācija (Chmielowiec-Korzeniowska et al., 2020). Pacienti, kuri iekļauti šajā pētījumā, bija jauni indivīdi (vidējais vecums 29 gadi)

bez tādiem *S. aureus* riska faktoriem kā HIV infekcija, insulīnatkarīgs cukura diabēts, pastāvīga peritoneālā vai hemodialīze, intravenozo narkotiku lietošana (Peacock et al., 2001; Chang et al., 2021; Ding et al., 2021; Wu et al., 2021). Arī citos pētījumos, kuros analizēti tonsilektomijas pacienti, vidējais vecums bija 28 gadi (Witsell et al., 2008; Senska et al., 2015).

Pētījumā uzmanība tika veltīta izdalīto *S. aureus* izolātu veidotās biofilmas novērtēšanai. *S. aureus* izolātiem, kas izdalīti no aukslēju mandeļu kriptām, novērotas izteiktākas biofilmas, salīdzinot ar *S. aureus* izolātiem, kas izdalīti no citām ķermeņa daļām. Biofilmas veidojošie *S. aureus* izolāti galvenokārt tika izdalīti no aukslēju mandeļu kriptām un bija jutīgi pret lielāko daļu testēto antibiotiku. *S. aureus* izdalītais viens variants tika identificēts kā MRSA, jo uzrādīja rezistenci pret cefoksitīnu. MRSA bija vājas biofilmas ražojošs izolāts. MRSA tika izolēts no 25 gadus vecas sievietes bez blakusslimībām, kura slimoja ar RT, pēdējo triju gadu laikā bija piecas tonsilīta epizodes. Pēc tonsilektomijas MRSA vairs nekonstatēja. MRSA RT pacientiem atklāts arī citos pētījumos. *Katkowska* et al. (2017, 2020) pētījumos Polijā MRSA izolāts tika izdalīts no aukslēju mandelēm vienam no 118 pieaugušajiem un diviem no 73 bērniem, kuriem veikta tonsilektomija (Katkowska et al., 2017, 2020). Biofilmu veidošanās un antibakteriālā rezistence MRSA un meticilīna jutīgais *S. aureus* (MSSA) ir neskaidrs (Senobar Tahaei et al., 2021). Vides faktori, piemēram, temperatūra, pH, glikozes līmenis, barotnes sastāvs u. c., ietekmē baktēriju biofilmu veidošanos. Tādēļ šie faktori jāņem vērā biofilmu pētījumos. Lai dažādu pētījumu rezultāti būtu savstarpēji salīdzināmi, vides faktoriem un biofilmu analīzes metodei jābūt vienādai vai ļoti līdzīgai (Liu et al., 2020). Ir nozīmīgi analizēt *S. aureus* kolonizāciju, lai izprastu to ierosinātās infekciju slimības. Pētījumā tika novērtēti RT pacienti un mikrobioloģiskās testēšanas rezultāti vienu gadu pēc tonsilektomijas. Mūsu pētījumā 10 pacientiem gadu pēc tonsilektomijas *S. aureus* netika konstatēts, tādēļ varam secināt, ka tonsilektomija novērš bakteriālu kolonizāciju viena gada periodā.

5.5. Darba kritiskais vērtējums

Cilvēku aukslēju mandeļu virsma un kriptas satur lielu patogēnu dažādību un ir augstā koncentrācijā. RT epizodes atkārtojas, neskatoties uz efektīvu konservatīvo terapiju akūtā periodā, kā arī var komplicēties par PTA. Pētījuma stiprās puses ir, ka pirmo reizi RT un PTA pacientu aukslēju mandeļu paraugi mikrobioloģiskai analīzei tika iegūti tonsilektomijas laikā ar biopsijas adatu, izslēdzot orālās mikrobiotas ietekmi. Veselo indivīdu paraugi, lai izvairītos no invazīvu manipulāciju veikšanas, tika iegūti ar birstīti no aukslēju mandeļu kriptām. Materiāla ņemšanas atšķirības ierobežoja triju pētījuma grupu savstarpējo salīdzināšanu.

Jāpiemin, ka veselie indivīdi bija statistiski nozīmīgi jaunāki, salīdzinot ar RT, PTA pacientiem, vidējais vecums attiecīgi 21 gads un 32 gadi. Pieejamā literatūrā nav datu par mikrobiotas atšķirībām pieaugušajiem šādā vecuma diapazonā. Pirmo reizi tika identificēti bakteriālie faktori, kuri varētu dot ieguldījumu skaidrojumam, kādēļ atkārtojas tonsilīta paasinājuma epizodes un dažos gadījumos attīstās smaga komplikācija – PTA. Pētījums nodrošina viena gada izsekojamības periodu ķirurģiski ārstētiem RT pacientiem. Pētījumā tika izvērtēta tonsilektomijas ietekme uz mikrobiotas klātbūtni un *S. aureus* nēsāšanu. Pacientu grupas lielumu ietekmēja vairāki faktori, piemēram, pēcoperācijas pacientu ierobežotās iespējas ierasties no dažādām Latvijas pilsētām uz atkārtotu vizīti Rīgā. PTA pacientu izlases lielumu ietekmēja PTA dažādās ārstēšanas metodes (pacienti, kuriem PTA ārstēšanā lietoja abscesa incīziju, drenāžu un terapiju ambulatori, netika iekļauti pētījumā).

Baktēriju identifikācijai tika izmantotas laboratorijas iekārtas *VITEK-2 Compact* vai *MALDI-TOF MS* sistēma. Baktēriju biofilmu veidošanos laboratorijas apstākļos ietekmē dažādi faktori, piemēram, temperatūra, pH, glikozes līmenis, barotnes veids. Pētījumā tika izmantota kristālvioletā metode, kas adaptēta pēc *Stepanović* et al., jo tā ir vienkārša, specifiska, ekonomiski izdevīga un bieži tiek lietota stafilokoku biofilmu kvantitatīvai noteikšanai (Stepanović et al., 2007). Svarīgi, ka šo metodi var viegli atkārtot citi pētnieki un pētījumu rezultātus var salīdzināt. Turpmākajos pētījumos ieteicams analizēt baktēriju ģenētiskos faktorus, kam ir nozīmīga loma biofilmas veidošanā. Šāda informācija ļautu precīzāk noteikt baktēriju lomu RT klīniskajā norisē un terapijas izvēlē.

5.6. Darba noslēdzošā rindkopa

Pirmo reizi aukslēju mandeļu kriptu parauga iegūšanai tika izmantota punktēšanas biopsijas adata, lai izslēgtu virspusējo orālās mikrobiotas baktēriju klātbūtni. Punktēšanas biopsijas adata tika patentēta.

Pētījumā tika izdalītas un identificētas RT un PTA pacientu aukslēju mandeļu kriptas kolonizējošās baktērijas, tika izvērtētas to īpašības, kurām varētu būt ietekme uz tonsilīta paasinājuma atkārtošanos un komplikāciju rašanos. Izvērtēta baktēriju biofilmu veidošanas spēja un tās saistība ar antibakteriālo rezistenci, kas ietekmē RT un PTA terapijas izvēli.

Tika pierādīts, ka pretēji pastāvošajiem uzskatiem *S. pyogenes* nav prevalējošais RT un PTA ierosinātājs, līdz ar to būtu jāpārskata streptokoku ātro testu un ASO noteikšanas nepieciešamība šiem pacientiem. Izplatītākais RT ierosinātājs pētītajā populācijā bija *S. aureus* (33,3 %, n = 33/99). PTA pacientu materiālā 48,28 % gadījumu (n = 14/29) tika identificēta *Candida* spp. Iespējams, ka *Candida* spp. klātbūtne var kalpot kā indikators PTA attīstībai. Tika

atrasts apstiprinājums arī izvirzītajai hipotēzei, ka RT un PTA gadījumā aukslēju mandeļu kriptās esošiem mikroorganismiem piemīt izteikta biofilmu veidošanas spēja, kas nodrošina hroniskas infekcijas klātbūtni ar iespējamu reaktivāciju. Antibakteriālās rezistences pētījumi liecināja, ka RT un PTA terapijai rekomendētās antibiotikas – benzilpenicilīns un ampicilīns – bija mazefektīvas, jo baktērijas uzrādīja rezistenci. RT un PTA etioloģiskie aģenti bija jutīgi pret amoksicilīnu ar klavulānskābi un klindamicīnu.

Pirmo reizi tika veikts plašs veselo indivīdu aukslēju mandeļu mikrobiotas pētījums. No veselu indivīdu aukslēju mandeļu materiāla 45 % indivīdu tika izdalīts *S. aureus*. Turklāt 95 % gadījumu (n = 39/41) tie bija biofilmu veidojošie izolāti. Veselu indivīdu gadījumā nenovēroja saistību starp biofilmas producēšanas spēju un antibakteriālo rezistenci. Gan *S. aureus* izolāti, gan citas biežāk izolētās baktērijas, *K. pneumoniae* un *P. aeruginosa*, bija jutīgas pret lielāko daļu antibiotiku.

Pirmo reizi tika izvērtēta ķirurģiskās un antibakteriālās ārstēšanas efektivitāte vienu gadu pēc tonsilektomijas. Uzsējumi no žāvas, deguna dobuma un padusēm liecināja, ka *S. aureus* tika izolēts retāk. *S. aureus* un to biofilmas veidošanas spējas noteikšana ir veids, kā identificēt *S. aureus* nēsātājus.

Secinājumi

1. RT pacientu aukslēju mandeļu kriptās ir liela mikroorganismu daudzveidība. *S. aureus* ir visizplatītākais patogēns, kas izolēts no RT pacientu aukslēju mandeļu kriptām, ar izteiktu biofilmu veidošanas spēju. *K. pneumoniae* biofilmas veidošanas pakāpe bija nenozīmīga. No PTA pacientu aukslēju mandeļu kriptām izdalītie baktēriju izolāti biofilmas veido retāk. *S. aureus* RT un PTA gadījumā ir jutīgi pret empīrisku antibakteriālu terapiju, ar izteiktu jutību pret amoksicilīnu ar klavulānskābi un klindamicīnu.
2. RT pacientu aukslēju mandeļu virsmas paraugos ir lielāka baktēriju daudzveidība salīdzinājumā ar kriptu biopsiju materiālu. Aukslēju mandeļu kriptu biopsija ar biopsijas adatu precīzāk atspoguļo RT ierosinātājus, jo izslēdz orālās mikrobiotas klātbūtni. No aukslēju mandeļu kriptām izdalītie *S. aureus* ir biofilmas veidojošie izolāti.
3. Veselu indivīdu aukslēju mandeles kolonizē *S. aureus, K. pneumoniae, P. aeruginosa* un *Acinetobacter* spp u. c. baktērijas. *S. aureus* ir biofilmu veidojošie izolāti. Aukslēju mandeļu kriptas ir nozīmīgs biofilmu producējošo patogēno baktēriju rezervuārs, kam var būt nozīme oportūnistisko tonsilītu etioloģijā.
4. No aukslēju mandeļu kriptām izdalītie *S. aureus* bija biofilmu veidojošie izolāti salīdzinājumā ar *S. aureus*, kuri izdalīti no deguna dobuma un padusēm. Vienu gadu pēc tonsilektomijas RT pacientiem *S. aureus* klātbūtne samazinājās. Tonsilektomija ir efektīva ārstēšanas metode, kura novērš bakteriālo kolonizāciju.

Publikāciju, ziņojumu un patentu saraksts par promocijas darba tēmu

Publikācijas (5):

1. **Viksne, R.**, Polikarpova, K., Jenbajeva, K. 2023. Evaluation of Tonsillectomy Patients and Factors Related to Immediate Tonsillectomy. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences.* (Izskatīšanas procesā.)
2. **Viksne, R.**, Racenis, K., Broks, R., Balode, A. O., Kise, L., Kroica, J. 2023. *In Vitro* Assessment of Biofilm Production, Antibacterial Resistance of *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Acinetobacter* spp. Obtained from Tonsillar Crypts of Healthy Adults. *Microorganisms*. 11. 258. https://doi.org/10.3390/microorganisms11020258
3. **Klagisa, R.**, Racenis, K., Broks, R., Balode, A. O., Kise, L., Kroica, J. 2022. Analysis of Microorganism Colonization, Biofilm Production, and Antibacterial Susceptibility in Recurrent Tonsillitis and Peritonsillar Abscess Patients. *Int. J. Mol. Sci.* 23. 10273. https://doi.org/10.3390/ijms231810273
4. **Klagisa, R.**, Racenis, K., Broks, R., Kise, L., Kroica, J. 2022. Evaluation of *Staphylococcus aureus* Colonization in Adult Patients Undergoing Tonsillectomy for Recurrent Tonsillitis. *Pathogens*. 11. 427. https://doi.org/10.3390/ pathogens11040427
5. **Klagisa, R.**, Kroica, J., Kise, L. 2021. *S. aureus* and *K. pneumoniae* on the Surface and within Core of Tonsils in Adults with Recurrent Tonsillitis. *Medicina*. 57. 1002. https:// doi.org/10.3390/medicina57101002

Patents (1):

1. **Klagisa, R.,** Kroica, J., Kise, L. 2021. Punktēšanas biopsijas adata. *Latvijas Republikas Patentu valdes oficiālais izdevums "Izgudrojumi, Preču Zīmes un Dizainparaugi".* 5. 315. ISSN 2255-9655. Patents Nr.: LVP2020000055

Referāti un tēzes starptautiskos kongresos un konferencēs (7):

1. **Viksne, R.,** Polikarpova, K., Jenbajeva, K. Evaluation of Tonsillectomy Patients and Factors Related to Immediate Tonsillectomy. *VIII Baltic ENT Congress*. Viļņa, Lietuva, 8.–10. jūnijs, 2023. (Mutisks ziņojums).
2. **Viksne, R.,** Racenis, K., Broks, R., Kroica, J. Bacterial Colonization and the Role of Bacterial Biofilms in the Upper Respiratory Tract. *Riga Stradiņš University International Conference on Medical and Health Care Science "Knowledge for Use in Practice".* Rīga, Latvija, 29.–31. marts, 2023. (Mutisks ziņojums).
3. **Klagisa, R.,** Kroica, J., Kise, L., Sumeraga, G., Asare, L. The Associations Between Bacteria, Fungi and Biofilm Production in Patients with Recurrent Tonsillitis and Healthy Controls. *Riga Stradiņš University International Conference on Medical and Health Care Science "Knowledge for Use in Practice".* Rīga, Latvija. 24.–26. marts, 2021. (Mutisks ziņojums).
4. **Klagisa, R.,** Kroica, J., Kise, L., Sumeraga, G., Asare, L. *Staphylococcus aureus* Colonisation in Patients with Recurrent Tonsillitis. *Riga Stradiņš University International Conference on Medical and Health Care Science "Knowledge for Use in Practice".* Rīga, Latvija. 24.–26. marts, 2021. (Stenda referāts).
5. **Klagisa, R.,** Balode, A. O., Broks, R., Kroica, J., Kise, L. Assessment of Biofilm Production by Pathogenic Bacteria Isolated from Tonsillar Crypts of Patients with Chronic Tonsillitis. *Riga Stradiņš University International Conference on Medical and Health Care Science "Knowledge for Use in Practice".* Rīga, Latvija. 1.–3. aprīlis, 2019. (Stenda referāts. Balva par labāko stenda referātu).

6. **Klagisa, R.,** Balode, A. O., Broks, R., Kroica, J., Kise, L. Microbiological Characteristics of Pathogenic Bacteria Isolated from Tonsillar Crypts of Patients with Chronic Tonsillitis. *Daugavpils Universitātes 61. starptautiskā zinātniskā konference.* Daugavpils, Latvija. 11.–12. aprīlis, 2019. (Mutisks ziņojums).
7. **Klagisa, R.,** Balode, A. O., Broks, R., Kroica, J., Kise, L. Microorganisms identified in tonsillar crypts of patients with chronic tonsillitis. *5th Congress of European ORL – HNS*. Brisele, Beļģija. 29. jūnijs – 3. jūlijs, 2019. (Stenda referāts).

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Analysis of Microorganism Colonization, Biofilm Production, and Antibacterial Susceptibility in Recurrent Tonsillitis and Peritonsillar Abscess Patients

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Abstract: Background: Despite the widespread use of antibiotics to treat infected tonsils, episodes of tonsillitis tend to recur and turn into recurrent tonsillitis (RT) or are complicated by peritonsillar abscesses (PTAs). The treatment of RT and PTAs remains surgical, and tonsillectomies are still relevant. Materials and methods: In a prospective, controlled study, we analyzed the bacteria of the tonsillar crypts of 99 patients with RT and 29 patients with a PTA. We performed the biofilm formation and antibacterial susceptibility testing of strains isolated from study patients. We compared the results obtained between patient groups with the aim to identify any differences that may contribute to ongoing symptoms of RT or that may play a role in developing PTAs. Results: The greatest diversity of microorganisms was found in patients with RT. Gram-positive bacteria were predominant in both groups. *Candida* species were predominant in patients with a PTA (48.3% of cases). Irrespective of patient group, the most commonly isolated pathogenic bacterium was *S. aureus* (in 33.3% of RT cases and in 24.14% of PTA cases). The most prevalent Gram-negative bacterium was *K. pneumoniae* (in 10.1% of RT cases and in 13.4% of PTA cases). At least one biofilm-producing strain was found in 37.4% of RT cases and in 27.6% of PTA cases. Moderate or strong biofilm producers were detected in 16 out of 37 cases of RT and in 2 out of 8 PTA cases. There was a statistically significant association found between the presence of Gram-positive bacteria and a biofilm-formation phenotype in the RT group and PTA group (Pearson χ^2 test, $p < 0.001$). *S. aureus* and *K. pneumoniae* strains were sensitive to commonly used antibiotics. One *S. aureus* isolate was identified as MRSA. Conclusions: *S. aureus* is the most common pathogen isolated from patients with RT, and *Candida* spp. are the most common pathogens isolated from patients with a PTA. *S. aureus* isolates are susceptible to most antibiotics. Patients with RT more commonly have biofilm-producing strains, but patients with a PTA more commonly have biofilm non-producer strains. *K. pneumoniae* does not play a major role in biofilm production.

Keywords: biofilm; recurrent tonsillitis; peritonsillar abscess

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1. Introduction

Recurrent tonsillitis (RT) is the repetitive inflammation of the palatine tonsils predominantly, or even exclusively, caused by bacteria [1,2]. Episodes of tonsillitis are characterized by fever, sore throat, odynophagia, congested tonsils with or without exudate, and cervical lymphadenopathy [1]. RT can be diagnosed clinically on an anamnestic report [3]. It can be considered when more than two distinct episodes of tonsillitis are encountered within a 12-month period [4]. Episodes of tonsillitis are treated with antibiotics [3,5]. Tonsillectomy is recommended for patients with RT who have experienced at least seven attack episodes

per year in the preceding one year, five episodes per year in the preceding two years, or three episodes per year in the preceding three years despite adequate antibiotic therapy [6].

Inflammation from the palatine tonsil can be transmitted to adjacent peritonsillar tissue and form an abscess in peritonsillar space [3]. A peritonsillar abscess (PTA) is the most common purulent complication of acute tonsillitis. The diagnosis of PTA is based on medical history combined with a general clinical assessment. A patient with a PTA typically presents with fever, a sore throat, unclear speech, sometimes trismus, or a reaction of the descending lymph nodes. Clinically, redness and arching of the palpably stiff and markedly painful soft palate are found [7]. A peritonsillar abscess is typically unilateral. A PTA requires antibiotics and surgical management—incision and drainage or immediate tonsillectomy must be performed [8].

The surgical removal of tonsils or tonsillectomy remains a common operation. For example, in Germany, in 2013, a total of 84,332 patients underwent extracapsular tonsillectomies, and approximately 12,000 surgical procedures in terms of abscess tonsillectomies or incision and drainage were performed for patients with a PTA [8]. Episodes of tonsillitis decrease the quality of life and are a financial burden due to school or work absences and health care costs [8]. The disadvantages of antibiotic therapy are the promotion of bacterial resistance and the surgical procedures that expose patients to surgical and anesthetic risks [8].

The explanations of unsuccessful antibiotic therapy are, for example, difficulty in identifying causative bacteria, low concentrations of the antibiotics in the tonsillar tissue, or the specific antibiotic resistance patterns of the involved pathogenic bacteria or biofilm formation [9,10]. Several studies have been conducted to elucidate the spectra of pathogens involved in RT and PTAs [2]. Several pathogens with varying proportions are implicated in tonsil infections, including group A beta hemolytic *Streptococcus*; alpha hemolytic *Streptococcus*, *Hemophilus influenzae*; *Staphylococcus aureus*; *Enterococcus* spp.; *Klebsiella pneumoniae*; *Moraxella catarrhalis*; *Corynebacterium* spp.; and anaerobes, such as *Peptostreptococci*, *Fusobacterium*, *Veillonella*, and *Prevotella* [3]. A microbiological analysis of tonsils is challenging due to the difficulties in distinguishing between commensal and pathogenic germs, great diversity, and differences in the normal microbiota between patients [3,11]. In previous studies, the tonsils were found to have the greatest microbial diversity, which varied significantly among subjects [12,13]. As cultures are obtained from an area that is normally heavily colonized, the etiological relevance of each bacterium is raised [14]. The tonsillar surface is colonized by a normal oral microbiota, which is not usually implicated in tonsil infections [15]. However, autoinfection via the normal flora of the mouth and the pharynx is also possible [3]. Tonsillar infection may stem from bacteria within tonsillar crypts or the parenchyma rather than from those on the surface [16]. Crypts are narrow passages that penetrate the tonsils. Therefore, samples from tonsillar crypts are considered more appropriate for microbiological testing than tonsillar surface swabbing [11,15,17].

Studies have been performed comparing the results of microbiological analyses between patients with recurrent tonsillitis and healthy subjects, but little differences were found between the study participants [18–21]. There are many studies that analyze the microbiological results of PTAs without comparisons with other patient groups [7,22–25]. Not so many studies concerning RT and PTA are available, and the reason for the lack of success of conservative therapeutic approaches is not well understood [2].

Studies show a high prevalence of *S. aureus* in tonsillar samples from patients with recurrent tonsillitis [2,26]. *S. aureus* is considered the main etiological factor of RT. However, the role of this pathogen in the pathogenesis of RT exacerbation, in the formation of abscesses, and in the resistance to antibacterial therapy, is unclear. As *S. aureus* does not show a high antibacterial resistance in RT, other protective mechanisms, such as biofilm-formation, should be considered. Biofilm-formation is thought to be associated with antibiotic tolerance [10]. In a previous study, scanning electron microscopy showed that biofilms were present in 80% (16/20) of the recurrent tonsillitis group and in 45% (9/20) of the control group [27]. The presence of biofilms was significantly higher in the recurrent

tonsillitis group, which suggests that biofilms are associated with recurrent tonsillitis [27]. The localization of the causative agents in biofilms could contribute to functional antibiotic resistance despite the absence of specific resistance mechanisms [2]. The primary problem in the treatment of patients with RT is usually difficulty in the effective eradication of the pathogen rather than its antibiotic resistance. *K. pneumoniae* is known to be a potent biofilm producer and has been isolated from tonsillar tissues [28,29].

In this study, emphasis is placed on the biological functions of bacteria in the tonsillar crypts of patients with RT and patients with a PTA. We provide a comprehensive analysis of the variety of isolated strains, *S. aureus* and *K. pneumoniae* biofilm formation, and antibacterial susceptibility with the aim of identifying any differences that may contribute to ongoing symptoms of RT or that may play a role in the development of PTAs.

2. Results

2.1. Patient Data

The age and gender ratios of the 99 patients with RT and the 29 patients with a PTA are listed in Table 1. According to our data, female patients predominated in the RT group, whereas the gender ratio was more balanced in the PTA group (Table 1). The RT group comprised 73 females (74%) and 26 males (26%), and the PTA group comprised 14 females (48%) and 15 males (52%). There was no age difference between the patients in the RT and PTA groups (independent-samples Kruskal–Wallis test, $p = 0.617$). The patients with a PTA showed increased white blood cell counts (WBCs) and C-reactive protein (CRP) levels in blood samples, and the patients with RT had WBCs and CRP levels within the normal range. These differences were highly significant.

Table 1. General characteristics of the study population.

Characteristics		RT	PTA	*p*-Value
Gender	Male, *n* (%)	26 (26%)	15 (52%)	$p = 0.061$
	Female, *n* (%)	73 (74%)	14 (48%)	$p = 0.061$
Age	Age (between, mean ± SD), years	20–72, 32.94 ± 11.19	18–58, 32.4 ± 12.2	$p = 0.279$
	Age (median, IQR), years	31, 10	31, 16	
Laboratory findings	CRP, median, mg/L	1.17	85.5	$p < 0.001$
	WBC, median, $\times 10^9$/L	6.52	12.97	$p < 0.001$
Comorbidities	Primary arterial hypertension (*n*)	9	1	$p = 0.454$
	Cardiologic diseases (*n*)	5	0	$p = 0.587$
	Type 2 diabetes mellitus (*n*)	1	0	$p > 0.999$
	Bronchial asthma (*n*)	5	1	$p > 0.999$
	Chronic gastritis or gastroesophageal reflux disease (*n*)	17	1	$p = 0.188$

2.2. Diversity of Isolated Microorganisms

Of the 128 patient samples examined, a positive cultivation finding (at least 1 pathogen or potential pathogen) was detected in 60 patients (60.6%) in the RT group and in 24 patients (82.8%) in the PTA group (Pearson χ^2 test, $p = 0.027$). However, the greatest diversity of microorganisms was found in the patients with RT. The cultivation finding was negative; i.e., only common oropharyngeal microbiotas were cultivated in 39 patients (39.4%) in the RT group and in 5 patients (17.2%) in the PTA group (Tables S1 and S2). Irrespective of patient group, the most commonly isolated pathogenic bacterium was *S. aureus*, which was isolated as the only microorganism or co-isolated with other potentially pathogenic microorganisms (Tables S1 and S2). In the RT group, *S. aureus* was isolated in 33.3% (33/99) of cases, and, in the PTA group, it was isolated in 24.14% (7/29) of cases. Gram-positive

bacteria were predominant, but at least one Gram-negative bacterium was detected in 22.2% (22/99) of patients in the RT group and in 27.6% (8/29) of patients in the PTA group. The most prevalent Gram-negative bacterium was *K. pneumoniae*; it was isolated in 10.1% (10/99) of RT cases and in 13.4% (4/29) of PTA cases (Tables S1 and S2). Moreover, *Candida* species were isolated, and they were predominant in patients with a PTA, where they were found in 48.3% (14/29) of cases and mostly as monocultures (Fisher's test, $p < 0.001$).

2.3. Biofilm Growth and Associations

At least one biofilm-producing strain was found in 37.4% (37/99) of cases of RT and in 27.6% (8/29) of cases of PTA (Pearson χ^2 test, $p = 0.332$). Moderate or strong biofilm producers were detected in 16 out of 37 cases of RT and in 2 out of 8 cases of PTA. In the RT group, among the 33 *S. aureus* isolates, 5 were strong, 8 were moderate, and 15 were weak biofilm producers, but 5 were biofilm non-producers. In the PTA group, among the 7 *S. aureus* isolates, 2 were weak biofilm producers, and 5 were biofilm non-producers. In the RT group, among the 10 *K. pneumoniae* isolates, 6 were weak biofilm producers, and 4 were biofilm non-producers. In the PTA group, among the 4 *K. pneumoniae* isolates, 1 was a weak biofilm producer, and 3 were biofilm non-producers. The biofilm mean optical densities (ODs) of all isolated *S. aureus* and *K. pneumoniae* strains are summarized in Figures 1 and 2.

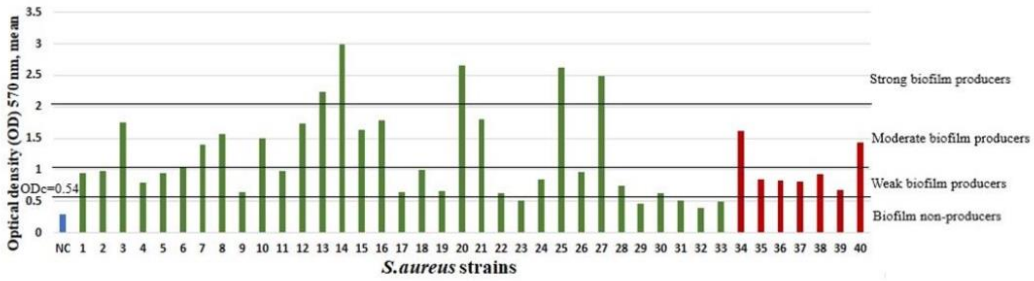

Figure 1. Biofilm-production capability on a microtiter plate of *S. aureus* strains from patients with RT (1–33 green bars) and patients with a PTA (34–40 red bars). Bars represent mean values of OD (measured at wavelength of 570 nm). Trypticase soy broth with 1% glucose was used as a negative control (NC, blue bar). The cut-off value (ODc) and biofilm-production-capacity levels are marked with horizontal lines.

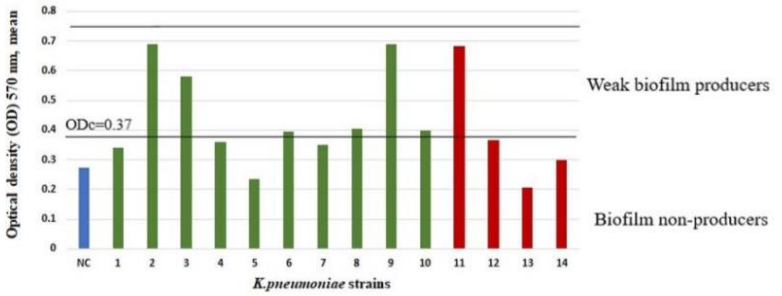

Figure 2. Biofilm-production capability on a microtiter plate of *K. pneumoniae* strains from patients with RT (1–10 green bars) and patients with a PTA (11–14 red bars). Bars represent mean values of OD (measured at wavelength of 570 nm). Luria–Bertani medium was used as a negative control (NC, blue bar). The cut-off value (ODc) and biofilm-production-capacity levels are marked with horizontal lines.

There was a statistically significant association found between the presence of Gram-positive bacteria and a biofilm-formation phenotype in the RT group and PTA group. If a Gram-positive microbe was present, there would most likely be a biofilm-formation phenotype (Pearson χ^2 test, $p < 0.001$). There were no significant associations found between Gram-negative microbes, *Candida* spp., comorbidities, episodes of tonsillitis, or PTAs in medical history and a biofilm-producing strain in the RT or PTA group. There was a tendency for the PTA group to have fewer biofilm-forming strains in comparison with the RT group, although statistically significant associations were not found between the presence of biofilm-producing strains or the presence of *S. aureus* biofilm-producing strains and patient group (Table 2).

Table 2. Comparison of patients' microbiological data in the RT and PTA groups.

Patients' Microbiological Data		RT Group	PTA Group	*p*-Values
Isolation rate	*S. aureus*, *n* (%)	33/99 (33.33%)	7/29 (24.14%)	$p = 0.347$
	K. pneumoniae, *n* (%)	10/99 (10.10%)	4/29 (13.79%)	$p = 0.519$
	Candida spp., *n* (%)	8/99 (8.08%)	14/29 (48.28%)	$p < 0.001$
Biofilms, mean OD	*S. aureus* biofilms, mean OD	1.24	1.02	$p = 0.929$
	K. pneumoniae biofilms, mean OD	0.44	0.39	$p = 0.322$
Biofilm-producing strains	Biofilm-producing strains, *n*	37	8	$p = 0.111$
	S. aureus biofilm-producing strains, *n*	28	7	$p = 0.642$
	S. aureus moderate and strong biofilm producers, *n* (%)	13/33 (39.39)	2/7 (28.57%)	$p = 0.691$
	K. pneumoniae moderate and strong biofilm producers, *n*	0	0	
Associations between variables by study groups	Gram-positive microbe and biofilm-producing strain	$p < 0.001$	$p < 0.001$	
	Gram-negative microbe and biofilm-producing strain	$p = 0.227$	$p > 0.999$	
	Candida spp. and biofilm-producing strain	$p > 0.999$	$p = 0.215$	
	Comorbidities and biofilm-producing strain	$p = 0.759$	$p = 0.540$	
	Episodes of tonsillitis and biofilm-producing strain	$p = 0.313$	$p = 0.738$	
	PTA in medical history and biofilm-producing strain	$p = 0.091$	$p = 0.640$	

2.4. Antibacterial Susceptibility

S. aureus and *K. pneumoniae* strains are sensitive to commonly used antibiotics. One *S. aureus* isolate was identified as MRSA, which is resistant to benzylpenicillin, ampicillin, cefoxitin, ceftriaxone, ampicillin–sulbactam, and amoxicillin with clavulanic acid, but has intermediate resistance to ciprofloxacin. *S. aureus* strains resistant to benzylpenicillin, ampicillin, and at least one other antibiotic, are shown in Tables 3 and 4 together with the biofilm-production capacity. Resistant strains are predominantly non-biofilm producers or weak biofilm producers. None of the *K. pneumoniae* isolates were extended-spectrum beta-lactamase producers.

Table 3. Antibiotic-susceptibility and biofilm-production-ability patterns of *S. aureus* strains.

Antibiotics	*S. aureus* Strains (*n* = 33) of Patients with RT			*S. aureus* Strains (*n* = 7) of Patients with a PTA		
	Resistant Strains (*n*)	Non- and Weak Biofilm Producers (*n*)	Moderate and Strong Biofilm Producers (*n*)	Resistant Strains (*n*)	Non- and Weak Biofilm Producers	Moderate and Strong Biofilm Producers
P, AMP, CIP *	20/33	12/20	8/20	5/7	4/5	1/5
P, AMP, CIP *, CD *	1/33	1				
CIP *	9/33	5/9	4/9	2/7	1/2	1/2
P, AMP, CIP *, E	1/33		1			
CIP *, E	1/33	1				
FOX, CRO, P, AMP, AMS, AUG, CIP *	1 **/33	1				

Note: *, intermediate resistance; **, MRSA; FOX, cefoxitin; CRO, ceftriaxone; P, benzylpenicillin; AMP, ampicillin; AMS, ampicillin–sulbactam; AUG, amoxicillin–clavulanic acid; CIP, ciprofloxacin; E, erythromycin; CD, clindamycin. Each antibiotic resistance was determined separately.

Table 4. Antibiotic-susceptibility and biofilm-production-ability patterns of *S. aureus* strains by patient group.

Patient Group	Biofilm Formation	Antibiotic Resistance (P, AMP)	No Antibiotic Resistance (or Antibiotic Resistance to One Antibiotic)	*p*-Value
RT group	Non- or weak biofilm producer	14	6	$p = 0.590$
	Moderate/strong biofilm producer	9	4	$p > 0.999$
PTA group	Non- or weak biofilm producer	4	1	$p > 0.999$
	Moderate/strong biofilm producer	1	1	$p > 0.999$
PTA + RT group	Non- or weak biofilm producer	18	7	$p = 0.590$
	Moderate/strong biofilm producer	10	5	$p > 0.999$

Note: P, benzylpenicillin; AMP, ampicillin. Each antibiotic resistance was determined separately.

3. Discussion

RT and PTAs are diseases with different clinical symptoms, disease courses, and prognoses. The common features of both diseases are as follows: they frequently occur among otolaryngology patients; their causative agent is most often a bacterium; and in cases of the ineffectiveness of antibacterial therapy, both diseases can be treated surgically. Comparably successful treatment regimens for both infections could be due to their similar etiologies [2].

Streptococcus pyogenes is the most common bacterial origin of acute tonsillitis in immunocompetent adults. While acute tonsillitis is postulated to only have one etiological factor, RT seems to have a multispecies etiology [5,30]. In our study, patients with RT had a great diversity of microorganisms, and polycultures were predominant. A similar finding has also been observed for other diseases. In comparison with healthy controls, increased microbial diversity is also associated with tuberculosis, cystic fibrosis, and gingivitis [12].

In our study, the isolation rate of *Streptococcus* spp. was low. Seventeen isolates of streptococci were identified in RT cases, and three were identified in PTA cases. *Streptococcus pyogenes* strains were co-isolated in four patients with RT, and none were isolated in patients with a PTA. Other studies have also reported a low isolation rate (1.7–5%) of streptococci in patients with RT; streptococci have been found to be less prevalent in the tonsillar core (1.7%) [29,31] than on the tonsillar surface. *S. pyogenes* in the RT pathogenesis has most likely been overrated or, alternatively, decreased in recent years [2,32].

The isolation rate of microorganisms varies depending on the approaches used for material collection. In a report by Zautner et al., *S. aureus* was prevalent in patients with

RT (57.7%), but *S. pyogenes* was prevalent in patients with a PTA (in 20.2% of tonsillar cell suspensions) [2]. In a study conducted by Vaikjarv et al., it was demonstrated that tonsillar fossa biopsy specimens were better materials for microbiological analyses than abscess pus samples, because they revealed more bacteria per culture [14]. *Streptococcus* spp. were the most common bacteria found in tonsillar fossa biopsy specimens and pus samples, but *Staphylococcus* spp. were also found in tonsillar fossa biopsy specimens, and staphylococci were not found in any pus cultures [14]. We chose to analyze biopsy samples to make the RT and PTA patient groups more comparable and inoculations more informative.

In our study, four *Streptococcus anginosus* isolates were found in the RT group, and none were found in the PTA group. In contrast, in another study, bacteria from the *Streptococcus anginosus* group were detected in the patient samples of the PTA renewal group more often than in those of the PTA recovery group, and the authors concluded that bacteria in the *Streptococcus anginosus* group appear to predict the renewal of PTA symptoms [33].

On the contrary, our study showed a high rate of *Candida* spp. They were isolated in 8.08% (n = 8) of patients with RT and in 48.23% (n = 14) of patients with a PTA. Several publications have claimed a lower isolation rate of *Candida* spp. In a report by Katkowska et al., *Candida* spp. were found in tonsillar core samples from patients with RT at a rate of 2.5%, in tonsillar surface samples at a rate of 8.3%, and in throat samples at a rate of 9.3% before tonsillectomy [5]. Zautner et al. reported *Candida* spp. in the tonsillar tissues of patients with RT at a rate of 12.8%, but, in patients with a PTA, this rate was 4.9% [2]. Slouka et al. reported yeasts in 2.3% of PTA pus aspirates [7]. Our study results are in agreement with those of a study conducted by Jokinen et al. [34], wherein fungal cultures of the tonsils of 147 patients with chronic tonsillitis revealed *Candida albicans* in 41.4% of cases; in the control group of healthy individuals, the rate of *Candida albicans* was 51–5% (34). The pathogenicity of the fungi was investigated in each case by histological means. The histological investigations revealed no evidence of pathogenicity in these organisms because they were found in the tonsillar crypts, and no granulomatous inflammation was seen surrounding them [34]. Another publication compared the palatine tonsil mycobiomes between individuals with human immunodeficiency virus (HIV) and those without it [35]. It was found that, between the individuals with HIV and those without it, in contrast to the bacteriomes, the palatine tonsil mycobiomes did not differ significantly between the two groups [35]. The role of *Candida* spp. in the tonsillar inflammatory process cannot be convincingly judged based on the results of our study, and it should be clarified in future studies.

In the scope of the present study, Gram-negative microorganisms were not the most important. *K. pneumoniae* is known to be a potent biofilm producer [28]. In our study, not only did *K. pneumoniae* have a low incidence, but it also showed low biofilm-producing ability. None of the *K. pneumoniae* isolates were a strong biofilm producer. *H. influenzae* is known to be more frequently isolated from the tonsillar core. In our study, it was isolated from two patients with RT, indicating that it is much less prominent than previously described. There was no statistically significant association between the presence of Gram-negative microbes and the presence of biofilm-producing strains in tonsillar tissues, in patients with RT, or in patients with a PTA.

In our study, the median age at the time of PTA occurrence was 31 years, which is in accordance with the results of other studies where patients were 29–34 years old [36,37]. In a previous study, an older population (over 40 years of age) with PTA was found to present with significantly lower rates of aerobic bacteria and a tendency toward higher rates of anaerobic growth; the authors clarified the need for prompt and aggressive surgical and antibiotic treatment for older patients with a PTA [38]. Group A *streptococcus* spp. was significantly more frequently recovered from patients with a PTA in the winter and spring than in the summer [22]. No seasonal differences were observed in our study. Blood of patients with PTA had higher WBCs and CRP levels compared to the blood samples of patients with RT. Patients with PTA were included in the study during the acute inflammation stage, which explains the high inflammatory parameters. Patients with RT had WBCs and CRP levels within normal range, but WBCs were at the lower limit of

normal range. RT patients may have changes in peripheral blood samples. Other studies had analyzed cytokine production, T and B lymphocytes, and the neutrophil-lymphocyte ratio in blood samples of patients with RT to draw reliable conclusions [39,40].

S. aureus isolates showed resistance to benzylpenicillin and ampicillin, which is consistent with the results of other studies. In a study conducted by Katkowska et al., *S. aureus* isolates from patients with RT showed resistance to penicillin in 79% of cases and to ampicillin in 63.2% of cases; only one *S. aureus* isolate was MRSA [5]. In our study, we did not observe associations between antibiotic resistance and biofilm-formation intensity. Even though several studies have claimed that intensive biofilm formation is associated with increased antibiotic tolerance, Ma et al. demonstrated that increased biofilm formation had the opposite effect and resulted in less antibiotic tolerance [41]. Our study data do not support the hypothesis that patients with a PTA are more likely to have strong biofilm-producing strains than other strains. On the contrary, in the PTA group, among the seven *S. aureus* isolates, two were weak biofilm producers, and five were biofilm non-producers. These findings suggest that *S. aureus* isolates could be wild-type strains and not an endogenous infection in the PTA group. Patients with RT who have PTAs in their medical history are more likely to have a biofilm-producing phenotype ($p = 0.091$). It is likely that, if a larger number of patients were included in this study, then the association would have been confirmed.

A biofilm is described as a bacterial community wrapped in a self-produced matrix of extracellular polymeric substances [42]. Studies have been carried out on the interactions between microorganisms. Interactions between *C. albicans* and *Staphylococcus* spp. are apparently synergistic or mutualistic, and they are increasingly reported [43]. The findings suggest that fungal cells can modulate the action of antibiotics and that bacteria can affect antifungal activity in mixed fungal–bacterial biofilms [44]. Prostaglandin E2 from *Candida albicans* stimulates the growth of *S. aureus* in mixed biofilms [45]. A chronic inflammatory process in palatine tonsils could be explained by the protected status of a bacterial community and a great variety of microorganisms.

4. Materials and Methods

A total of 128 patients were enrolled in this prospective, monocentric study. A total of 29 patients were diagnosed with PTAs, and 99 patients had RT. Study patients underwent tonsillectomy in Pauls Stradins Clinical University Hospital in the period of 2018–2020.

The inclusion criteria were as follows: a diagnosis of PTA or RT, bilateral tonsillectomy received as surgical treatment, and no antibacterial therapy for at least 4 weeks. RT was defined as at least seven attack episodes per year in the preceding one year, five episodes per year in the preceding two years, or three episodes per year in the preceding three years despite adequate antibiotic therapy. PTA was defined clinically by redness and arching of the palpably stiff and markedly painful soft palate and fever, a sore throat, unclear speech, sometimes trismus, and a reaction of the descending lymph nodes. All RT patients underwent scheduled tonsillectomy, with the last episode of tonsillitis being more than 4 weeks ago. All PTA patients underwent immediate tonsillectomy under acute infection stage.

The exclusion criteria were as follows: hematologic system diseases (thrombocytopenia or coagulopathy), primary or acquired immunodeficiency, antibacterial therapy received in the last 4 weeks or the initiation of antibacterial treatment prior to material collection, outpatient treatment, other methods of PTA treatment such as incision and drainage, and failure to perform tonsillectomy (contraindications for surgery or general anesthesia) or to obtain written informed consent. Patients with active solid or hematological malignancy, active autoimmune disease, usage of immunosuppressive agents prior to admission (prednisone > 10 mg/day or equivalent) more than 4 weeks, and solid organ transplant, were excluded.

Pediatric patients were not excluded from the study since only adult patients (18 years old or older) are treated at Pauls Stradins Clinical University Hospital. Pediatric patients receive medical treatment at the Children's Clinical University Hospital in Riga, Latvia.

After tonsillectomy, a histological analysis of the specimens from all patients was routinely performed to verify the clinical diagnosis.

The study protocol was approved by the local Ethics Committee of Riga Stradins University (document No. 49/30.11.2017.), and all of the data were collected according to the guidelines on data protection and confidentiality. Written informed consent was obtained from all subjects before the study.

4.1. Sample Collection

Samples for microbiological testing were obtained from tonsillar crypts with a punch-biopsy needle perioperatively [17,46]. The samples contained specimens of affected tonsillar tissue in the case of a PTA.

4.2. Isolation of Microorganisms and Microbiological Investigation

Punch-biopsy samples of tonsillar crypts were taken, placed in Amies transport media, and transported at room temperature within 24 h to the laboratory. The samples were cultivated on Columbia blood agar, Mannitol salt agar, MacConcey agar, and Sabouraud dextrose agar plates at 36 ± 1 °C for 24–48 h aerobically. A brucella blood agar plate in an anaerobic pouch system, incubated at 36 ± 1 °C for up to five days, was used for the cultivation of anaerobes. A Columbia can blood agar plate with an optochin disc incubated in a CO_2 incubator at 36 ± 1 °C for 24–48 h was used for the cultivation of Streptococcus pneumoniae, and a chocolate agar plate with an oleandomycin disc incubated in an CO_2 incubator at 36 ± 1 °C for 24–48 h was used for the cultivation of *Haemophylus* spp.

We took note of the common oropharyngeal microbiota as described by the European Society of Clinical Microbiology and Infectious Diseases [47]. The identification of the considered pathogens was performed using a Microflex LT (Bruker Daltonics flexAnalysis version 3.4, Bruker Daltonics GmbH & Co. KG, Bremen, Germany) matrix-assisted laser desorption ionization–time-of-flight mass spectrometer (MALDI–TOF MS) system.

Susceptibility testing and the evaluation of the results were performed using the disc diffusion method, and the evaluation of the results was carried out according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard actual EUCAST version [48].

4.3. Biofilm Growth Using Cristal Violet Assay

Isolated Gram-positive strains were suspended in trypticase soy broth (TSB) supplemented with additional 1% glucose, and Gram-negative strains were suspended in Luria–Bertani (LB) broth for incubation at 37 °C for 16–18 h. Inoculated broths were diluted with sterile TSB or LB broths in a ratio of 1:100. Then, 150 µL of the diluted suspension was transferred with a multichannel pipette in sterile 96-well plates (Thermo Scientific™ Nunc MicroWell 96-Well Microplates, flat bottom, Thermo Fisher Scientific, Roskilde, Denmark). Each plate contained 11 strains, and the negative control (uninoculated broth) contained 8 wells per strain; each experiment was performed in triplicate. The inoculated plates were cultivated aerobically at 37 °C for 48 h. After incubation, all wells were emptied by gently throwing out the liquid in a clinical waste bag without the use of a pipette. Each well was rinsed 3 times with sterile 250 µL 0.9% saline. After washing, staining was performed by adding 150 µL of 0.1% crystal violet per well. After 15 min, the color was removed by gently throwing out the color, and each well was washed 3 times with 250 µL distilled water. At the end, 150 µL of 96% ethanol was added to each well. Afterwards, the optical densities (ODs) of the wells were measured at a 570 nm wavelength with a microplate spectrophotometer (Tecan Infinite F50, Mannedorf, Switzerland, with Magellan™ reader control and data analysis software V 6.6) [49].

4.4. Biofilm Calculation

The OD values for each strain were averaged and are expressed as numbers. The cut-off value (ODc) was defined as three standard deviations (SDs) above the mean OD of the negative control, and it was separately calculated for each plate. Strains were divided as follows: OD $\leq$ ODc = no biofilm producer, ODc < OD $\leq$ 2 $\times$ ODc = weak biofilm producer, 2 $\times$ ODc < OD $\leq$ 4 $\times$ ODc = moderate biofilm producer, and 4 $\times$ ODc < OD = strong biofilm producer [50].

4.5. Statistical Analysis

A statistical analysis was performed using IBM SPSS Statistics version 26 (Chicago, IL, USA) and Microsoft Excel 10. For all of the hypotheses tested, a *p*-value of less than 0.05 indicated statistical significance.

5. Conclusions

5.1. Conclusions

S. aureus is the most common pathogen isolated from patients with RT, and *Candida* spp. are the most common pathogens isolated from patients with a PTA.

S. aureus isolates associated with RT and PTAs are susceptible to most antibiotics.

Patients with RT more commonly have biofilm-producing strains, but patients with a PTA have biofilm-non-producer strains as causative agents.

K. pneumoniae does not play a major role in biofilm production.

5.2. Strengths and Limitations

Several limitations should be addressed. Firstly, we analyzed microorganisms separately, and it would be desirable to analyze their interactions, as well as the functional aspects of the biofilm. Secondly, further studies are necessary to evaluate the role of *Candida* spp. *S. aureus* surface proteins and to genotype the pathogenesis of tonsillitis.

6. Patents

Klagisa, R.; Kroica, J.; Kise, L. Punch Biopsy Needle. Patent No: LVP2020000055. In *Izgudrojumi, Preču Zīmes un Dizain-paraugi*. Patent Office of the Republic of Latvia, Riga, Latvia, 2021; Volume 5, p. 315.

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S. aureus and *K. pneumoniae* on the Surface and within Core of Tonsils in Adults with Recurrent Tonsillitis

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Abstract: *Background and Objectives*: Recurrent tonsillitis is an infection of the palatine tonsils. Samples for microbiological testing are usually obtained from the inflamed surface of the tonsils. Colonizing the surface bacteria does not always correlate with pathogens causing recurrent tonsillitis and there is no consensus or this in research studies. The aim of the study was to compare whether *Staphylococcus aureus* (*S. aureus*) and *Klebsiella pneumoniae* (*K. pneumoniae*) differ when isolated from the tonsillar surface or tonsillar crypts in patients with recurrent tonsillitis. *Materials and Methods*: a case series study was conducted at a tertiary referral center among 25 patients diagnosed with recurrent tonsillitis. An evaluation of *S. aureus* and *K. pneumoniae* incidence, biofilm formation and antibacterial susceptibility was performed. *Results*: There was a statistically significant association between surface and punch biopsy samples for *S. aureus* (Fisher's Exact test $p = 0.004$) and *K. pneumoniae* (Fisher's Exact test $p < 0.001$). A McNemar test did not reveal a statistically significant association. Although the antibacterial resistance profile was not broad, five out of nine *S. aureus* isolates were biofilm producers and four out of five *K. pneumoniae* isolates were biofilm producers. *Conclusions*: Surface and core cultures of tonsils are comparable with a differing incidence between the surface and the punch biopsy cultures for *S. aureus* and *K. pneumoniae*. A larger quantity of bacteria exist in surface samples suggesting that a biopsy sample may be less challenging in evaluating recurrent tonsillitis. We recommend that antibacterial susceptibility results are considered alongside the biofilm-forming potential of isolated bacteria.

Keywords: *Klebsiella pneumoniae*; *Staphylococcus aureus*; surface and core; tonsillitis

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1. Introduction

Recurrent tonsillitis is an infection of the palatine tonsils, which is characterized by recurrent episodes of tonsillitis, resulting in continuous discomfort in the throat, collections of debris in the palatine crypts, halitosis and cervical lymphadenopathy. Acute symptoms (episodes of tonsillitis) are induced by bacteria released from tonsillar crypts [1] Chronic symptoms are caused by bacteria sheltered within the tonsillar crypts leading to prolonged interaction with the host's immune system [1].

Samples for microbiological testing are usually obtained from the inflamed surface of the tonsils by rotating sterile cotton wool swabs over the surface, avoiding any other part of the oropharynx [2]. Only superficial microorganisms that are highly variable are analyzed and contamination with the normal oral microbiota cannot be ruled out [3]. Colonizing surface bacteria do not always correlate with pathogens causing recurrent tonsillitis and there is no consensus for this in research studies [2,4,5]. Patients' microbiota can be affected by recent hospitalization or travelling, concomitant diseases or the use of antibiotics. The living environment of patients is important due to variable microbiological changes associated with urban and rural living environments. The study by Khadilkar and Ankle (2016) showed that anaerobic organisms known to inhabit the surface were

also present at the core of tonsils and the same antibiotics were efficient against both the surface and core bacteria [5]. The time frame for the acquisition of clinical specimens is limited due to pathogenic microorganisms being present on the surface of tonsils only during exacerbations of tonsillitis. It is not possible to obtain the contents of crypts with a cotton swab. Crypts are narrow passages that penetrate the tonsils. There are microbes in the depth of the crypts. It is recommended to take the clinical specimen from the upper pole of the palatine tonsil, where the largest crypt (*crypta magna* in Latin) is located and where the most common complication, peritonsillar abscess, occurs [6]. Tonsillar infection may stem from bacteria within tonsillar crypts or parenchyma rather than from those on the surface [5].

The objective of this study is to compare whether *Staphylococcus aureus* (*S. aureus*) and *Klebsiella pneumoniae* (*K. pneumoniae*) differ when isolated from the tonsillar surface or tonsillar crypts in patients with recurrent tonsillitis.

2. Materials and Methods

Case series study was performed in Pauls Stradins Clinical University Hospital (PSCUH) in Riga, Latvia, from August 2020 to September 2020. The study protocol was approved by the Ethics Committee of Riga Stradins University (document No. 49/30.11.2017.). Written informed consent was obtained from all subjects before the study. Those with recent antibacterial treatment or those who had failed to give consent were excluded. There was no control group as the samples were taken from patients with a known history of recurrent tonsilitis that were referred to the Otorhinolaryngology Department of PSCUH for scheduled tonsillectomy. A brush sample from uninflamed tonsillar surface was taken prior to surgery and a punch biopsy of tonsillar crypts was performed during surgery in 25 adults undergoing tonsillectomy for recurrent tonsillitis.

For research purposes, the punch biopsy needle was designed with a prolonged and curved handle and a circular blade for tonsillar crypt biopsy (patent number: LVP2020000055) [7].

The brush samples and punch biopsy samples were inoculated on mannitol salt agar and MacConkey agar for *S. aureus* un *K. pneumoniae* to be isolated. The isolated bacteria were identified using VITEK-2 Compact (bioMériux, France).

Microtiter-plate method was used for in vitro cultivation and quantification of bacterial biofilms [8]. The optical density of the layer of adherent biofilm formed in the microtiter-plate was measured using a microtiter-plate reader (Tecan Infinite F50, Mannedorf, Switzerland, with Magellan™ reader control and data analysis software V 6.6).

Antibacterial susceptibility tests were performed, and the results were evaluated in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 'Clinical breakpoints and dosing of antibiotics' (Version 10.0, January 2020) [9]. Overnight cultures were suspended in physiological saline to 0.5 McFarland units (McFarland Densitometer DEN-1, Biosan, Latvia). The suspension was inoculated on Mueller-Hinton agar (Oxid, UK). Selected antibiotics were placed on the inoculated plates and included ceftazidime 10 μg, ampicillin 10 μg, cefotaxime 5 μg, meropenem 10 μg, imipenem 10 μg, amikacin 30 μg, ciprofloxacin 5 μg, chloramphenicol 30 μg, amoxicillin + clavulanic acid 30 μg, and piperacillin + tazobactam 36 μg (Liofilchem, Italy).

3. Results

Twenty-five patients were included in this study that comprised of four male and 21 female patients. They were aged between 20 and 71 years, with a median age of 31 at the time of data collection. Seventeen patients were from Riga, three patients from Jelgava, and five patients from other cities (Engure, Bauska, Salaspils, Limbazi and Tukums) in Latvia.

Sixteen patients had concomitant diseases: one patient had bronchial asthma; one patient had a renal abscess and nephrectomy in their medical history; one patient had psoriasis; one patient had pheochromocytoma; three patients had primary arterial hypertension (PAH); one patient had migraine and cystitis in their medical history; one patient

had gastritis; one patient had gastroesophageal reflux disease (GERD); one patient had GERD and dysregulation of glucose metabolism; one patient had PAH, Graves' disease and myocarditis in their medical history; one patient had PAH and uterine fibroids; one patient had GERD, Lyme disease and gout; one patient had tick-borne encephalitis in their medical history; one patient had polyarthritis and hepatitis; and nine patients denied any concomitant diseases.

Twenty-one patients had had recurrent episodes of tonsillitis during the past 3 years, four patients had recurrent episodes of tonsillitis during childhood and had chronic discomfort in the throat, tonsilloliths and halitosis, as adults. Two patients had peritonsillar abscess and one patient had peritonsillitis in their medical history. Four patients had had cryptolysis with radiofrequency, three patients had had cryotherapy before surgery. The most recent antibacterial treatment was received 1 week and 2 years before surgery, with a median time of 6 months. Four patients were abroad 3 weeks to 2 months before the scheduled tonsillectomy. Ten patients were hospitalized (one in January 2020, two in 2018, four in 2017, one in 2016, one in 2013, one in 2008), one patient worked in the hospital as medical staff member, and one patient worked at a cattle breeding farm.

C-reactive protein was tested in 18 patients and was between 0.1 and 6 mg/L with a median of 0.82 mg/L. The white blood cell count was tested in 25 patients and was between 3080 and 9150, with a median of 5760. Five patients had anti-streptolysin O tested, which obtained the measurements 68.1, 277, 96.29, 44.95 and 90 Iu/mL. The rheumatoid factor was tested in six patients, which obtained the measurements 19, 7.8, 11.06, 19, 6.9, and 3. The erythrocyte sedimentation rate was tested in 14 patients, and was measured as 2 and 25 with a median of 3.

S. aureus was isolated from brush samples of 12 patients and from the punch biopsy specimens of nine patients (Table 1). Fisher's Exact test revealed a statistically significant association between *S. aureus* isolation from brush and punch biopsy samples ($p = 0.004$). *K. pneumoniae* was isolated from the brush samples of four patients from the punch biopsy specimens of five patients (Table 1). Fisher's Exact test revealed a statistically significant association between *K. pneumoniae* isolation from brush and punch biopsy samples ($p < 0.001$). A McNemar test did not reveal a statistically significant association.

Table 1. Growth of *S. aureus* and *K. pneumoniae* from brush and punch biopsy samples.

	Brush Only	Punch Only	Both	Brush Total	Punch Total	Test, p Value	
S.aureus	4	1	8	12 (25)	9 (25)	Fisher's Exact test, $p = 0.004$	McNemar test, $p = 0.375$
K.pneumoniae	0	1	4	4 (25)	5 (25)	Fisher's Exact test, $p < 0.001$	McNemar test, $p > 0.999$

S. aureus isolates detected in punch biopsy were tested for their biofilm-producing ability (Table 2). Five out of nine *S. aureus* isolates were biofilm producers, two isolates were strong biofilm producers, three isolates were weak biofilm producers, but four out of nine *S. aureus* isolates did not produce a biofilm. Four out of five *K. pneumoniae* isolates were weak biofilm producers and one out of five *K. pneumoniae* isolates were biofilm non-producers.

Table 2. Biofilm production and antibacterial susceptibility profile of *S. aureus* and *K. pneumoniae* isolates obtained with punch biopsy from tonsils of patients undergoing tonsillectomy.

	Biofilm Production		Antibacterial Susceptibility			
	Biofilm Producers	Biofilm Non Producers	Benzylpenicillin	Ampicillin	Ciprofloxacin	Erythromycin
S.aureus	5 (9)	4 (9)	R 7 (9)	R 7 (9)	I 9 (9)	0 (9)
K.pneumoniae	4 (5)	1(5)	R 5 (5)	R 5 (5)	0 (5)	R 5 (5)

S. aureus and *K. pneumoniae* isolates detected in punch biopsy samples were tested for antibacterial susceptibility. Seven out of nine *S. aureus* isolates were resistant to benzylpenicillin and ampicillin; five out of seven were biofilm producers. All *S. aureus* isolates were intermediate to ciprofloxacin, and sensitive to ampicillin-sulbactam, amoxicillin-clavulanic acid, amikacin, erythromycin, clindamycin, and chloramphenicol. All *K. pneumoniae* isolates were resistant to benzylpenicillin, ampicillin and erythromycin, but sensitive to ampicillin-sulbactam, amoxicillin-clavulanic acid, piperacillin-tazobactam, ceftazidime, cefotaxime, ceftriaxone, meropenem, imipenem, amikacin and ciprofloxacin.

There were no significant associations between recurrent episodes of tonsillitis and concomitant diseases (Fisher's exact test, $p = 0.542$); recurrent episodes of tonsillitis and the presence of *S. aureus* in punch biopsy samples (Fisher's exact test, $p = 0.260$), or the presence of *K. pneumoniae* in punch biopsy samples (Fisher's exact test, $p > 0.999$); recurrent episodes of tonsillitis and biofilm production of *S. aureus* (Fisher's exact test, $p = 0.238$), or biofilm production of *K. pneumoniae* (Fisher's exact test, $p = 0.617$); and recurrent episodes of tonsillitis and resistance of *S. aureus* (Fisher's exact test, $p = 0.294$), or the resistance of *K. pneumoniae* (Fisher's exact test, $p > 0.999$) (Table 3.).

Table 3. Characteristics of the study population. Note: PAH, primary arterial hypertension; GERD, gastroesophageal reflux disease; DGM, dysregulation of glucose metabolism; RT, recurrent episodes of tonsillitis during the past 3 years; SA, *S. aureus*; KP, *K. pneumoniae*; 0, biofilm nonproducer; 1, weak producer; 2, moderate producer; 3, strong producer; R, resistance; BP, benzylpenicillin; AMP, ampicillin; E, erythromycin.

N	Age	Sex	Place	Concomitant Diseases	RT	*SA* Brush	*SA* Punch	*KP* Brush	*KP* Punch	*SA* Biofilm	*KP* Biofilm	*SA* Resistance (BP, AMP)	*KP* Resistance (BP, AMP, E)
1	31	♀	Riga	Bronchial asthma	+	+	+	-	-	3		R	
2	27	♀	Engure	Renal abscess, nephrectomy	+	+	+	-	-	1		R	
3	30	♀	Bauska	Psoriasis	+	-	-	+	+		1		R
4	30	♀	Riga	Pheochromocytom	+	-	+	-	-	3		R	
5	20	♀	Jelgava		+	-	-	-	-				
6	64	♀	Jelgava	PAH	-	+	-	-	-				
7	31	♀	Riga		-	-	-	-	-				
8	26	♀	Riga		+	+	+	-	-	1		R	
9	32	♀	Riga	Migraine, cystitis	+	-	-	-	-				
10	36	♀	Riga		+	+	+	-	-	0		R	
11	31	♀	Riga		+	-	-	-	-				
12	26	♀	Salaspil		+	+	+	-	-	1		R	
13	31	♂	Riga	Gastritis	+	-	-	-	+		0		R
14	25	♀	Riga	GERD, DGM	+	-	-	+	+		1		R
15	71	♀	Riga	PAH, Graves' disease, myocarditis	+	+	-	-	-				
16	61	♀	Jelgava	PAH, uterine fibroids	+	-	-	-	-				
17	55	♂	Riga	GERD, Lyme disease, gout	-	-	-	+	+		1		
18	34	♀	Limbazi		+	-	-	-	-				
19	22	♀	Riga		+	+	+	-	-	0			
20	55	♀	Riga	PAH	-	-	-	-	-				
21	40	♀	Riga		+	-	-	-	-				
22	38	♂	Tukums	Tick-borne encephalitis	+	+	+	+	+	0	1		
23	27	♂	Riga	PAH	+	+	+	-	-	0			
24	41	♀	Riga	Polyarthritis, hepatitis	+	+	-	-	-				
25	58	♀	Riga	GERD	+	+	-	-	-				

Tonsillectomy specimens were sent for routine histopathological examination. The histopathological evaluation reports were reviewed and the diagnosis was chronic nonspecific tonsillitis in all patients.

4. Discussion

The median age of patients in this study was 31; they were older compared to previous studies, in which patients were predominantly adolescents aged 11 to 20 (44%), followed by children (41%) [5], with the median age being 24 [3]. This study was carried out in Pauls Stradins Clinical University Hospital where only adult patients (≥18 years old) were admitted, therefore explaining the differences in patients' age. Our study, like other studies before [3,5] had predominantly female participants. In a study by Khadilkar and Ankle, which included 100 patients of chronic tonsillitis, female predominance was explained with an increased health awareness in women [5].

The microbiota of patients and the *S. aureus* carriage can be affected by factors such as working on a farm, recent hospitalization or travelling, concomitant diseases and use of antibiotics. At the time of sampling, patients did not receive antibacterial treatment.

The histories of patients' concomitant diseases were taken and cross referenced with medical records prior to surgery. At the time of surgery, no exacerbations of chronical illnesses were noted. Some patients' medical histories showed infectious diseases such as renal abscesses, tick-borne encephalitis, Lyme disease, cystitis, hepatitis, and myocarditis in the past. Inflammatory markers (white blood cell count, C-reactive protein, and erythrocyte sedimentation rate) were within normal range. The specific onset of concomitant diseases in relation to recurrent tonsillitis was not clear due to the lack of specific medical records of such nature and patients' failure to recall their medical history.

An incidence of surface and core isolates was observed, and an accuracy of culture findings was compared in many studies [2,3,5,10,11]. The accurate identification of the bacterial organism responsible for an infected tonsil might improve culture-directed antibiotic therapy and obviate the need for elective tonsillectomy [11]. In our study we analyzed *S. aureus* as studies proved that *S. aureus* persisted within mucosal biofilms, even intracellularly [12–15]. *K. pneumoniae* is well known for its biofilm-forming potential. *K. pneumoniae* biofilms can lead to colonization in the respiratory tract, nasopharynx, tonsils [16,17]. In a study by Sarkar et al. (2017), *S. aureus*, group A beta-hemolytic streptococci, and *Klebsiella spp.* were the most common isolates from both surface and core samples with a higher incidence in core samples [11]. Sarkar et al. (2017) concluded that the routine culture of surface swab specimens in patients with recurrent tonsillitis was neither reliable nor valid and recommended core sampling using fine needle aspiration as the diagnostic method of choice [11]. In the current study, the growth of *S. aureus* was more common in brush samples, whereas *K. pneumoniae* was isolated more frequently from punch biopsy samples.

For punch biopsy isolates we provided biofilm growth testing and antimicrobial susceptibility testing. Although the antibacterial resistance profile was not broad, the biofilm growth testing revealed that five out of nine *S. aureus* isolates and four out of five *K. pneumoniae* isolates were biofilm producers. Microorganisms in biofilms were distinctively more resistant to antimicrobial agents and environmental insults and were therefore more difficult to eradicate [1].

5. Strengths and Limitations

The punch biopsy needle was developed specifically to obtain the core samples of the tonsils. The report provides the results of the microbiological testing of *S. aureus* and *K. pneumoniae* on the surface and within the core of tonsils in adults with recurrent tonsillitis. Microbiological testing provides information of biofilm forming ability of identified bacteria.

However, several limitations should be addressed. Firstly, the small number of cases observed during the study period could cause bias. Due to the relatively small sample

size, we focused mainly on the presence of *S. aureus* and *K. pneumoniae* in tonsillar samples obtained in different ways. Further studies, including those with a larger study group, a control group, an increased bacterial spectrum with biofilm formation and antibacterial susceptibility tests would be necessary to draw more reliable conclusions in terms of tonsillitis. Additionally, a histopathological study of punch biopsy samples would be useful to measure the presence of inflammatory cells and perform a cell count of them. Then, these variables could be compared with the presence of bacteria.

6. Conclusions

There was a statistically significant association between the surface and punch biopsy samples for *S. aureus* and *K. pneumoniae*. The surface and core cultures of tonsils were comparable with a differing incidence between the surface and punch biopsy cultures for *S. aureus* and *K. pneumoniae*. A larger quantity of bacteria existed in surface samples suggesting that a biopsy sample may be less challenging in evaluating recurrent tonsillitis. We recommend that antibacterial susceptibility results are considered alongside the biofilm forming potentials of isolated bacteria.

7. Patents

Klagisa, R.; Kroica, J.; Kise, L. Punch Biopsy Needle. Patent No: LVP2020000055. In *Izgudrojumi, Preču Zīmes un Dizain-paraugi*. Patent Office of the Republic of Latvia: Riga, Latvia, 2021; Volume 5, pp. 315.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee) of Riga Stradins University (protocol code 49/30.11.2017. and date of approval 30 November 2017).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author, through the institutional review board. The data are not publicly available due to restrictions of the institution.

Conflicts of Interest: The authors declare no conflict of interest.

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In Vitro Assessment of Biofilm Production, Antibacterial Resistance of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. Obtained from Tonsillar Crypts of Healthy Adults

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Abstract: Background and Objective: Tonsillar crypts can be considered a reservoir for a variety of bacterial species. Some bacterial species can be considered part of the normal oropharyngeal microbiota. The roles of other pathogens, for example, the so-called non-oral and respiratory pathogens *Staphylococcus aureus*, *Klebsiella*, *Pseudomonas*, and *Acinetobacter* spp., which have strong virulence factors, biofilm production capacity, and the ability to initiate infectious diseases, are unclear. The purpose of this study was to detect the presence of *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp. within the tonsillar crypts of healthy individuals, and to analyze the pathogens' biofilm production and antibacterial resistances. Results: Only common oropharyngeal microbiota were cultivated from 37 participant samples (40.7%). The most commonly isolated pathogenic bacterium was *S. aureus*, which was isolated in 41 (45%) participant samples. *K. pneumoniae* was isolated in seven (7.7%) samples, *Acinetobacter* spp. were isolated in five (5.5%) samples, and *P. aeruginosa* was isolated in two (2.2%) samples. Biofilm producers predominated among the pathogenic bacteria; 51 strains were biofilm producers, and among them, 31 strains were moderate or strong biofilm producers. The tested *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp. strains were sensitive to commonly used antibiotics (amoxicillin–clavulanic acid, clindamycin, or ciprofloxacin). One of the isolated *S. aureus* strains was MRSA. Conclusions: Biofilm is a commonly observed feature that seems to be a naturally existing form of pathogenic bacteria colonizing human tissue. *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp. occasionally occur in the tonsillar crypts of healthy individuals, and, therefore, it is most likely that *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp. in opportunistic tonsillar infections originate from the tonsillar crypt microbiota.

Keywords: biofilm; colonization; tonsillar microbiota; tonsillar crypts

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1. Introduction

All mucosal surfaces of the human body are colonized by a plethora of bacterial communities [1]. Different oral structures and tissues are colonized by distinct microbial communities [2]. Oral microbiota have been shown to be functionally connected to infectious and inflammation-related diseases [3]. The palatine tonsils are mucosa-associated and immunocompetent lymphoid organs localized on the lateral wall of the oropharynx [3]. They are continuously exposed to bacteria from saliva, inhaled air, ingested food, and the airway surface liquids of the respiratory tract, and play an essential role in the human immune defense system via surveillance, detection, and the initiation of an immune response [4]. Both the surface of the tonsils and the extensive tubular tonsillar crypts are

important colonization sites for many pathogenic and commensal microorganisms [5]. Tonsillar infections may stem from bacteria within the tonsillar crypts or the parenchyma, rather than from those on the surface [6]. The microbiota of the palatine tonsils play an important role in health through the etiology of infection and the carriage of adventitious pathogens.

The oropharyngeal microbiome has been extensively characterized through cultivation and culture-independent molecular methods [1–3]. Oropharyngeal bacterial communities are dominated by six major phyla, *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Spirochetes*, and *Fusobacteria*, representing 96% of all taxa found in the oropharynx [1,2,7]. Less dominant taxa are highly specific to both individuals and body habitats [8]. In the oral cavity, most habitats are dominated by *Streptococcus*, and these are followed in abundance by *Haemophilus* in the buccal mucosa, *Actinomyces* in the supragingival plaque, and *Prevotella* in the subgingival plaque [8]. Less dominant taxa, species that pose a modest degree of risk, and various clinically important pathogens which are generally considered non-oral bacteria, such as Gram-negative enteric rods, enterococci, and staphylococci, are highly important [9]. Disease states are often associated with a disruption of the microbial community, frequently resulting in one or a few pathogenic organisms emerging. *Staphylococcus (S.) aureus* is of particular interest as the cause of methicillin-resistant *S. aureus* (MRSA) infections, as are the respiratory bacterial pathogens *Klebsiella (K.) pneumoniae*, *Pseudomonas (P.) aeruginosa*, and *Acinetobacter* spp., which are potent biofilm producers ([9–11], p. 2). The pathogens mentioned above are among the ESKAPE pathogen group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) and have been declared critical priority pathogens by the World Health Organization due to their increasing levels of resistance to commonly used antibiotics [12].

Knowledge regarding biofilms has significantly increased over the years, from the attachment of biofilms to artificial surfaces to mucosal biofilms. Studies have revealed that mucosal biofilms exist in both healthy and diseased individuals, and that the presence of a mucosal biofilm is not always associated with disease [13]. Biofilms may exist in the palatine tonsils of healthy adults due to cryptic tissue structure, a temperature lower than physiological body temperature, and direct, repeated exposure to respiratory bacterial pathogens [10]. Signs that differentiate between "healthy" and "pathological" biofilms are currently being sought [10].

Biofilms play a role in the process of chronic and recurrent infections due to certain important pathology-associated features of biofilms, including enhanced resistance to antibiotic treatments and increased host defense [14]. Biofilm-associated bacteria can be up to 1000 times more resistant to antimicrobial agents relative to planktonic bacteria [10,13]. Mucosal biofilm has been implicated in relation to recurrent tonsillitis [15].

Based on our hypothesis that tonsillar crypts are richly colonized and covered with bacterial biofilm even in the absence of disease, the aim of this study was to detect the presence of the so-called non-oral and respiratory pathogens *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp. within the tonsillar crypts of healthy medical students, and to analyze the pathogens' biofilm production and antibacterial resistance. Medical students are present in medical institutions more often than the general public, so there is a higher likelihood of them being carriers of pathogenic strains.

2. Materials and Methods

A total of 91 healthy students from the Medical Faculty of Riga Stradins University were included in a prospective cohort study from 1 October 2019 to 31 December 2019. The healthy individuals were fifth-semester Medical Faculty students for whom otolaryngology was the first clinical subject taught at the hospital, and they had not yet been exposed to patients. The inclusion criteria were absence of tonsillar pathologies or upper respiratory tract infections at the time of data collection, no comorbidities, and no antibacterial therapy for at least 4 weeks. Those who received antibacterial therapy in the last 4 weeks, had

unhealthy oral conditions (including tooth decay and periodontal disease), had prosthetic devices within the oral cavity, or who failed to give consent were excluded.

The study protocol was approved by the local ethics committee of Riga Stradins University (document no. 49/30.11.2017), and all the data were collected according to the relevant guidelines on data protection and confidentiality. Written informed consent was obtained from all subjects before the study.

2.1. Sample Collection

Samples for microbiological testing were obtained from tonsillar crypts using a brush (Kito brush, reference number 0640, Kaltek srl, Padova, Italy).

2.2. Isolation of Microorganisms and Microbiological Investigation

Material from tonsillar crypts were taken, placed, and transported in AMIES transport medium at room temperature within 24 h and cultivated on two Columbia blood agar plates with and without optochin disk, Brucella blood agar, Chocolate agar with oleandomycin disc, Mannitol salt agar, MacConkey agar, and Sabouraud dextrose agar plates. Columbia blood agar, Mannitol salt agar, MacConkey agar, and Sabouraud dextrose agar plates were incubated at 36 ± 1 °C for 24–48 h aerobically. A Brucella blood agar plate was incubated in a BD GasPak™EZ pouch system at 36 ± 1 °C for up to five days. A Columbia blood agar plate with an optochin disc incubated in a CO_2 incubator at 36 ± 1 °C for 24–48 h was used for the cultivation of *Streptococcus pneumoniae*. A Chocolate agar plate with an oleandomycin disc incubated in a CO_2 incubator at 36 ± 1 °C for 24–48 h was used for the cultivation of *Haemophylus* spp. We took note of the common oropharyngeal microbiota as described by the European Society of Clinical Microbiology and Infectious Diseases [16]. Microorganisms that are not part of the common oropharyngeal microbiota were considered as potential pathogens. The identification of the considered pathogens was performed using a Microflex LT (Bruker Daltonics flex Analysis version 3.4, Bruker Daltonics GmbH & Co. KG, Bremen, Germany) matrix-assisted laser desorption ionization–time-of-flight mass spectrometer (MALDI–TOF MS) system.

2.3. Antibacterial Susceptibility Testing

Susceptibility testing was performed by the Kirby–Bauer disk diffusion method. Overnight cultures were suspended in physiological saline to 0.5 McFarland units (McFarland Densitometer DEN-1, Biosan, Latvia). The suspension was inoculated on Mueller–Hinton agar (Oxid, UK). Selected antibiotics were placed on the inoculated plates. For *S. aureus* strains, cefoxitin 30 µg, ceftriaxone 30 µg, benzylpenicillin 1iu, ampicillin 2 µg, ampicillin–sulbactam 10/10 µg, amoxicillin–clavulanic acid 20/10 µg, norfloxacin 10 µg, amikacin 30 µg, erythromycin 15 µg, clindamycin 2 µg, and chloramphenicol 30 µg were applied (Liofilchem, Italy). For *K. pneumoniae* and *Serratia liquefaciens* strains, amoxicillin–clavulanic acid 20/10 µg, piperacillin–tazobactam 30/6 µg, cefotaxime 5 µg, ceftazidime 10 µg, ertapenem 10 µg, imipenem 10 µg, meropenem 10 µg, ciprofloxacin 5 µg, gentamicin 10 µg, and trimethoprim/sulfamethoxazole 1.25/23.75 µg were applied (Liofilchem, Italy). For *Acinetobacter* spp., piperacillin–tazobactam 30/6 µg, ceftazidime 10 µg, imipenem 10 µg, meropenem 10 µg, ciprofloxacin 5 µg, and amikacin 30 µg were applied (Liofilchem, Italy). For *Acinetobacter* spp., imipenem 10 µg, amikacin 30 µg, gentamicin 10 µg, trimethoprim/sulfamethoxazole 1.25/23.75 µg, ciprofloxacin 5 µg, and levofloxacin 5 µg were applied (Liofilchem, Italy). The size of the zone of inhibition around the disk was measured after 16–20 h of incubation. The evaluation of the results was carried out according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard, actual EUCAST version [17].

2.4. Biofilm Growth Using Crystal Violet Assay

Isolated Gram-positive strains were suspended in trypticase soy broth (TSB) supplemented with additional 1% glucose, and Gram-negative strains were suspended in Luria–Bertani (LB) broth for incubation at 37 °C for 16–18 h. Inoculated broths were diluted with sterile TSB or LB broths at a ratio of 1:100. Then, 150 µL measures of the diluted suspensions were transferred with a multichannel pipette into sterile 96-well plates (Thermo Scientific™ Nunc MicroWell 96-Well Microplates, flat bottom, Thermo Fisher Scientific, Roskilde, Denmark). Each plate contained 11 strains, and the negative control (uninoculated broth) contained 8 wells per strain; each experiment was performed in triplicate. The inoculated plates were cultivated aerobically at 37 °C for 48 h. After incubation, all wells were emptied by gently throwing out the liquid into a clinical waste bag without the use of a pipette. Each well was rinsed three times with sterile 250 µL 0.9% saline. After washing, staining was performed by adding 150 µL of 0.1% crystal violet per well. After 15 min, the color was removed by gently throwing out the color, and each well was washed three times with 250 µL distilled water. Finally, 150 µL of 96% ethanol was added to each well. Afterwards, the optical densities (ODs) of the wells were measured at a wavelength of 570 nm using a microplate spectrophotometer (Tecan Infinite F50, Mannedorf, Switzerland, with Magellan™ reader control and data analysis software V 6.6) [18].

2.5. Biofilm Calculation

The OD values for each strain were averaged and expressed as numbers. The cut-off value (ODc) was defined as three standard deviations above the mean OD of the negative control and was separately calculated for each plate. Strains were divided as follows: $OD \leq ODc$ = biofilm nonproducer, $ODc < OD \leq 2 \times ODc$ = weak biofilm producer, $2 \times ODc < OD \leq 4 \times ODc$ = moderate biofilm producer, and $4 \times ODc < OD$ = strong biofilm producer [19].

2.6. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 26 (Chicago, IL, USA) and Microsoft Excel 10 (Microsoft, Redmond, WA, USA). For all of the hypotheses tested, a *p*-value of less than 0.05 indicated statistical significance.

3. Results

3.1. Patient Data

The study group included 52 females (57%) and 39 males (43%) aged between 19 and 29 years (mean, 21.2 ± 1.41 years, median, 21 years).

3.2. Diversity of Isolated Microorganisms

Of the 91 participant samples examined, a positive cultivation finding (at least one pathogen or potential pathogen) was detected in 54 participant samples (59.3%) (Table 1). The cultivation finding was negative, i.e., only common oropharyngeal microbiota were cultivated, in 37 participant samples (40.7%) (Table 1). The most commonly isolated pathogenic bacterium was *S. aureus*, which was isolated as the only microorganism or co-isolated with other potentially pathogenic microorganisms in 41 participant samples (45%) (Table 1). Gram-positive bacteria were predominant, but at least one Gram-negative bacterium was detected in 16 samples (17.6%). Among the Gram-negative bacteria, *K. pneumoniae* was the most common, and it was isolated in seven samples.

Table 1. Microorganisms isolated from tonsillar crypts of 91 healthy individuals.

Combinations of Isolated Strains	Count (n)
Normal oral microbiota	37
S. aureus + normal oral microbiota	20
S. aureus	16
S. aureus + *Acinetobacter junii*	2
S. aureus + *K. pneumoniae*	1
S. aureus + *Candida* spp. + *Streptococcus viridans*	1
S. aureus + *K. pneumoniae* + *Serratia liquefaciens* + normal oral microbiota	1
K. pneumoniae	5
P. aeruginosa	2
Acinetobacter pittii	2
Acinetobacter johnsonii	1
Serratia liquefaciens	1
Streptococcus dysgalactiae	1
Acinetobacter ewofii + normal oral microbiota	1

3.3. Biofilm Growth and Associations

Forty-one (41) *S. aureus* strains and fifteen strains of Gram-negative bacteria were tested for biofilm production. *S. aureus* strains were predominantly biofilm producers: 25 out of 41 (61%) *S. aureus* strains were moderate or strong biofilm producers, and 14 out of 41 (34.1%) *S. aureus* strains were weak biofilm producers, but 2 out of 41 (4.9%) *S. aureus* strains were biofilm nonproducers (Figure 1). Among the Gram-negative bacteria, 6 out of 15 (40%) strains were moderate or strong biofilm producers and 6 out of 15 (40%) strains were weak biofilm producers, but 3 out of 15 (20%) strains were biofilm nonproducers (Figure 2).

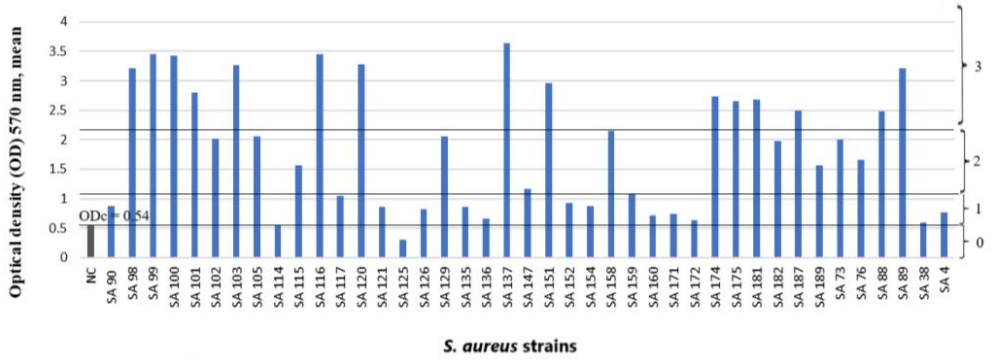

Figure 1. Biofilm production capability on microtiter plate of 41 *S. aureus* strains. Bars represent mean values of OD (measured at wavelength of 570 nm). Trypticase soy broth with 1% glucose as a negative control (NC). The number designates the participant; the letters indicate the strain isolated: SA—*Staphylococcus aureus*. The cut-off value (ODc) and biofilm production capacity levels are marked with horizontal lines: 0—biofilm nonproducers; 1—weak biofilm producers; 2—moderate biofilm producers; 3—strong biofilm producers.

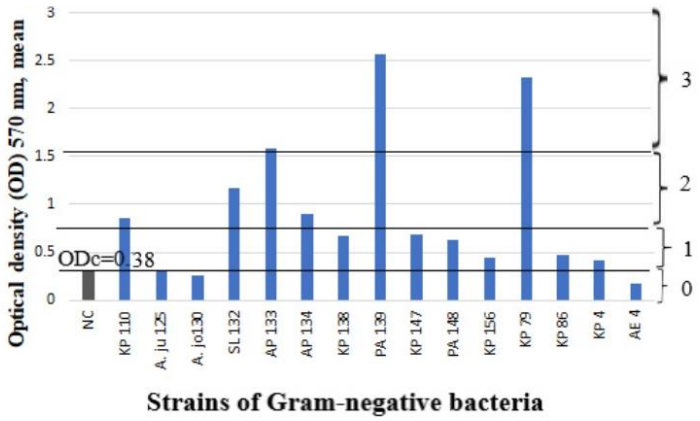

Figure 2. Biofilm production capability on microtiter plates of 15 strains of Gram-negative bacteria. Bars represent mean values of OD (measured at wavelength of 570 nm). Luria–Bertani medium as a negative control (NC). The number designates the participant; the letters indicate the strain isolated: KP—*Klebsiella pneumoniae*; A.ju—*A.junii*; A.jo—*Acinetobacter johnsoni*; SL—*Serratia liquefaciens*; AP—*Acinetobacter pitti*; PA—*Pseudomonas aeruginosa*; and AE—*Acinetobacter ewoffi*. The cut-off value (ODc) and biofilm production capacity levels are marked with horizontal lines: 0—biofilm nonproducers; 1—weak biofilm producers; 2—moderate biofilm producers; 3—strong biofilm producers.

A summary of the study participants' microbiological data is shown in Table 2. There was a statistically significant association found between the presence of Gram-positive bacteria and a biofilm-formation phenotype. If a Gram-positive microbe was present, there would most likely be a biofilm-formation phenotype (Pearson $\chi 2$ test, $p < 0.001$) (Table 2).

Table 2. Summary of microbiological data of study participants.

Participants' Microbiological Data		Results	*p*-Values
Isolation rate	Normal oral microbiota only, n (%)	37/91 (40.7%)	
	Gram-positive strains, n	43	
	Gram-negative strains, n	17	
Biofilms, mean OD	*S. aureus* biofilms, mean OD	1.89	
	Gram-negative microbe, mean OD	0.95	
Biofilm-producing strains	Biofilm-producing strains, n	51	
	S. aureus biofilm-producing strains, n	39	
	Gram-negative microbe biofilm-producing strains, n	12	
	Strong and moderate biofilm producers, n	31	
Associations between variables	Gram-positive microbe and biofilm-producing strain		$p < 0.001$
	Gram-negative microbe and biofilm-producing strain		$p = 0.808$

3.4. Antibacterial Susceptibility

The tested *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp. strains were sensitive to commonly used antibiotics, for example, amoxicillin–clavulanic acid, clindamycin, or ciprofloxacin (Tables 3 and 4). Gram-negative rods were sensitive to all antibiotics tested (Table 4). Only one *Acinetobacter junii* strain was resistant to amikacin. One of the isolated *S. aureus* strains was methicillin-resistant *S. aureus* (MRSA), which was

resistant to cefoxitin. It was a strong biofilm producer. None of the isolated *K. pneumoniae* strains were extended-spectrum beta-lactamase (ESBL) producers. No statistically significant correlations were noted between the antibiotic susceptibility pattern and the biofilm production capacity.

Table 3. Antibiotic resistance among *S. aureus* strains isolated from healthy subjects.

		Antibiotic Resistance (%)										
	Strains (n)	**FOX**	**CRO**	**P**	**AMP**	**AMS**	**AUG**	**NOR**	**AK**	**E**	**CD**	**C**
S. aureus	41	2.4	2.4	75.6	75.6	2.4	2.4	2.4	2.4	14.6	0	4.9

FOX, cefoxitin; CRO, ceftriaxone; P, benzylpenicillin; AMP, ampicillin; AMS, ampicillin–sulbactam; AUG, amoxicillin–clavulanic acid; NOR, norfloxacin; AK, amikacin; E, erythromycin; CD, clindamycin; C, chloramphenicol.

Table 4. Antibiotic resistance among *K. pneumoniae*, *P. aeruginosa*, *Serratia liquefaciens*, and *Acinetobacter* spp. strains isolated from healthy subjects.

		Antibiotic Resistance (%)										
	Strains (n)	**AUG**	**TZP**	**CTX**	**CAZ**	**ETP**	**IMP**	**MEM**	**CIP**	**GM**	**SXT**	**AK**	**LEV**
K. pneumoniae	7	0	0	0	0	0	0	0	0	0	0		
P. aeruginosa	2		0		0		0	0	0			0	
Acinetobacter spp.	5						0		0	0	0	20	0
Serratia liquefaciens	1	0	0	0	0	0	0	0	0	0	0		

AUG, Amoxicillin–clavulanic acid; TZP, Piperacillin—tazobactam; CTX, Cefotaxime; CAZ, Ceftazidime; ETP, Ertapenem; IMP, Imipenem; MEM, Meropenem; CIP, Ciprofloxacin; GM, Gentamicin; SXT, Trimethoprim-sulfamethoxazole; AK, Amikacin; LEV, Levofloxacin.

4. Discussion

The oropharynx provides heterogeneous niches for bacterial colonization. Since tonsillar infection may stem from bacteria within tonsillar crypts or the parenchyma rather than from those on the surface, we focused on the microbiota in tonsillar crypts as the most critical region for the development of tonsillopathies [1,6]. In our study of healthy individuals, we isolated and analyzed such non-oral and respiratory pathogens as *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp. [9,10].

The primary ecological niche for *Staphylococcus* is the nostrils; nevertheless, the oral cavity comprises a significant reservoir for these bacteria, and some adults exhibit exclusive oral colonization [20]. In a study by Albrich and Harbarth, the colonization of extranasal sites was associated with the persistent carriage of *S. aureus* [21]. In a study carried out in the USA by Hanson and colleagues, it was reported that 6.2% of adults carried *S. aureus* only in the anterior nares, 18.6% only in the oropharynx, and 19.8% in both sites [22]. A carrier rate in the oral cavity from 17 to 48% has been reported within student populations [23,24]. Healthy Swedish dental students had an *S. aureus* prevalence of 44.6%; no MRSA was detected among them [24]. Healthcare workers were found to carry MRSA at a rate of 23.7% [21]. The prevalence of MRSA in healthy carriers has been reported to range from 1.5 to 26% [25–27]. In our study, *S. aureus* was the most common pathogen isolated; it was isolated in 45% of cases, and MRSA was isolated in 1.1% of cases, which is in accordance with previous studies.

In a study by Jeong and colleagues, *K. pneumoniae* was isolated from the tonsillar core samples of recurrent tonsillitis patients in 6.7% of cases, and from those of tonsillar hypertrophy patients in 1.5% of cases [28]. Our study showed that *K. pneumoniae* was present in the tonsillar crypt specimens of healthy subjects in 7.7% of cases.

Several studies have analyzed the role of extracellular or intracellular *P. aeruginosa* in the origins of periodontal or pulmonary diseases [29,30]. In one study, *P. aeruginosa* was the third most common pathogen after *E. faecalis* and *S. aureus* in human buccal and gingival epithelial cells obtained from subjects with periodontitis and periodontally healthy subjects;

no difference was observed in the prevalence of *P. aeruginosa* between periodontitis and periodontally healthy subjects or between the types of epithelial cells [31]. In another, *P. aeruginosa* was detected at high mean prevalence and counts in the subgingival microbiota and was closely related to periodontal inflammation and tissue destruction [32]. Other studies have reported a 1.4–3.8% prevalence of *P. aeruginosa* in the tonsillar samples of recurrent tonsillitis patients [28,33,34], and a 0.9% prevalence in the tonsillar samples of tonsillar hypertrophy patients [28]. Our study showed that *P. aeruginosa* was present in the tonsillar crypt specimens of healthy subjects in 2.2% of cases. Among *Acinetobacter* strains, *Acinetobacter baumannii* was not detected. The variety and prevalence of pathogen isolation from tonsil samples may vary depending on the sampling method; for example, tonsil surface swabs may be less informative than tonsil crypt material [35].

Tonsillar crypts are a suitable site for biofilm formation. Tonsillar crypts are able to collect debris, and the mineralization of this debris leads to tonsillolith formation [36]. Tonsilloliths possess dynamic biofilms similar to dental biofilms [37]. Our study showed that in healthy subjects, 61% of *S. aureus* strains and 40% of Gram-negative bacteria strains were moderate or strong biofilm producers. Our study confirms that biofilm formation is a normal bacterial lifestyle, and that biofilms can exist in the tonsils of healthy individuals. In the study by Penesyan and colleagues, biofilm was described as a main microbial lifestyle; biofilms perform an important function for microbes by providing a protective environment in which genotypic and phenotypic diversity is generated before being released [38]. Biofilm characteristics may differ between diseased and healthy individuals. Chervinets and colleagues reported that the microbiota of the oral cavities of patients with periodontitis had a greater ability to adhere to the cells of the mucous membrane than those of healthy people, while their ability to form biofilms and exhibit pathogenic properties was enhanced [39].

The localization of the causative agents in biofilms may contribute to antibiotic resistance. Antibiotic resistance is a major concern regarding *S. aureus*, especially MRSA. An increasing prevalence of MRSA in healthy carriers has been reported, amounting up to 21% in the nasal samples of dental students [25]. The prevalence of MRSA in the oral cavity is less known; subgingival sites and tongue surfaces were tested in a previous study, and no MRSA was detected [40]. Our study revealed one (1.1%) MRSA isolate from tonsil specimens. An important finding in this study was the high rate of benzylpenicillin- and ampicillin-resistant *S. aureus* strains isolated from healthy individuals; however, no isolates were resistant to clindamycin. The data that were obtained about *S. aureus* antibacterial resistance are in accordance with the study by Katkowska et al. [41]. Clindamycin is widely used in dentistry, and many clinics have substituted it for common penicillins (oxacillin and methicillin); clindamycin is prescribed in the case of allergy to beta-lactams [24].

It has been hypothesized that infectious strains have different virulence arsenals than those colonizing healthy individuals [24]. However, some studies have failed to show, for example, that *S. aureus* strains isolated from oral infections and noninfected controls represent different subgroups of phenotypic and genotypic characteristics [24]. It has therefore been suggested that classical opportunistic infections develop due to an imbalance within the host–parasite relationship, and that the infectious disease persists as long as the compromised condition prevails [24].

We would like to highlight certain strengths of the present study. We used tonsil brushes as an alternative, noninvasive method for collecting tonsil specimens, eliminating the need to traumatize tonsils in order to collect tonsil tissue. Therefore, in our study, we included healthy individuals without any signs of palatine tonsil disease. The limitations of this study were the absence of a comparison group, a small number of Gram-negative bacterial strains analyzed, and in vitro biofilm formation. The environmental factors (for example, temperature, pH, glucose level, type of media) influence bacterial biofilm production. We created the best possible conditions for bacterial growth and biofilm formation in vitro as described by Stepanović et al. [19]. However, complex in vivo models for biofilm studies are superior and encouraged. In further studies, it is recommended

to analyze bacterial genetic factors as well, as they also play an important role in biofilm formation and would be useful for such an analysis.

5. Conclusions

Biofilm is a commonly observed feature that seems to be a naturally existing form of pathogenic bacteria colonizing human tissue. *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp. occasionally occur in the tonsillar crypts of healthy individuals, and, therefore, it is most likely that *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp. in opportunistic tonsillar infections originate from the tonsillar crypt microbiota.

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Evaluation of *Staphylococcus aureus* Colonization in Adult Patients Undergoing Tonsillectomy for Recurrent Tonsillitis

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Abstract: Background and objectives: *Staphylococcus aureus* (*S. aureus*) is often recovered from the pharynx. However, the role of this pathogen in the etiology of tonsillar inflammation is still unclear and complicated due to frequent carriage of *S. aureus*. The aim of the study was to evaluate the frequency and the clinical importance of *S. aureus* colonization and biofilm production ability in patients with recurrent tonsillitis (RT) using patient samples from tonsillar crypts during tonsillectomy, and from the throat, nasal cavity, and armpits after tonsillectomy. Materials and Methods: A case series study was carried out at a tertiary referral center among 16 patients diagnosed with RT who were undergoing tonsillectomy. Samples from tonsillar crypts were obtained during tonsillectomy, and samples from the throat, nasal cavity, and armpit were obtained a year after surgery. An evaluation of *S. aureus* incidence, biofilm formation, and antibacterial susceptibility was performed. Results: During tonsillectomy, 16 strains of *S. aureus* were isolated from 16 patients, while 15/16 *S. aureus* strains were biofilm producers. A year after tonsillectomy, 8 *S. aureus* strains were isolated from 6 out of 16 patients, while 6/8 *S. aureus* strains were biofilm producers. After tonsillectomy, 3 patients showed *S. aureus* in throat culture. Conclusions: In 10/16 cases *S. aureus* was the causative agent of RT, in 4/16 cases patients had a predisposition to colonization of *S. aureus*, and in 2/16 cases *S. aureus* was a part of the patients' oral microbiome. Tonsillectomy results in less frequent isolation of *S. aureus* strains.

Keywords: biofilm; carrier; colonization; recurrent tonsillitis; *Staphylococcus aureus*

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1. Introduction

The ecological niche of *Staphylococcus aureus* (*S. aureus*) in humans is the anterior nares, however it can be frequently isolated from the throat, palatine tonsils, and skin [1]. Approximately 20–25% of the healthy adult population have become persistently colonized by *S. aureus*, 60% intermittently, while 20–30% of population are non-carriers [2]. Hanson et al. (2018) reported that in the USA, from 177 adults, 6.2% carried *S. aureus* only in the anterior nares, 18.6% only in the oropharynx, and 19.8% in both sites [3]. They found out that the prevalence of oropharyngeal carriage was higher in urban (47.3%) than rural (27.9%) environments [3]. Chmielowiec-Korzeniowska et al.'s (2020) study demonstrated that every third adult (32%) in Poland was an asymptomatic carrier of *S. aureus*, and *S. aureus* was recovered from the pharynx more often than from the nose or skin [2].

Previous studies have shown that there is a strong causal connection between *S. aureus* nasal carriage and increased risk of nosocomial infection in dialysis patients and in those undergoing surgery [1,4]. *S. aureus* is an etiological factor of such purulent infections as furuncles, abscesses, osteomyelitis, and sepsis. However, the role of this pathogen in the etiology of tonsillar inflammation is still unclear and complicated due to frequent carriage of *S. aureus*. Aside from *S. aureus*, other aerobic and anaerobic pathogens are also implicated

in tonsil infections, for example alpha and beta hemolytic Streptococcus (group A, C), *Hemophilus influenzae*, *Haemophilus parainfluenzae*, *Enterococcus* spp., *Klebsiella pneumoniae*, *Corynebacterium* spp., *Peptostreptococci*, Fusobacteria, *Bacteroides*, and *Veillonella* [5–7]. Oral microbiota, which are potential pathogens, may make it difficult to identify the causative agent of tonsillitis. Researchers claim that routine culture of surface swab specimens in patients with recurrent tonsillitis (RT) is not reliable and recommend core sampling as the diagnostic method of choice [6,8,9].

Regardless of the widespread use of antibiotics to treat infected tonsils, episodes of tonsillitis tend to recur and form a condition known as recurrent tonsillitis (RT). Episodes of tonsillitis decrease quality of life and are a financial burden due to absences in school or work and health care costs [6]. RT can be treated with tonsillectomy in those patients in whom at least five or more attack episodes occur in a year [5,10]. If less than three episodes are observed up to the time of first presentation, the indication for surgery cannot be made until at least six episodes occur within the observation period [10].

Importantly, *S. aureus* strains can acquire broad antibacterial resistance. Parts of *S. aureus* strains isolated from patients with RT were considered multidrug-resistant and methicillin-resistant (MRSA) [11,12]. In the Netherlands, healthcare workers who are MRSA carriers achieved successful MRSA eradication only after tonsillectomy [13]. Antibiotic resistance can be explained by inadequate penetration of antibiotics into the tonsillar core, the protection of bacteria within epithelial cells and macrophage-like cells, the resistance of strains to the typical antibiotic treatments due to repeated antibiotic courses, and the prevalence of biofilm-producing bacteria (5,6). According to Brook and Foote's (2006) study, *S. aureus* strains are more often found in the tonsillar core than on the tonsillar surface [14]. *S. aureus* persistence in tonsillar tissues is still a matter of discussion and requires further research [15].

The ability of *S. aureus* to produce multilayered, mature biofilms may contribute to the survival of *S. aureus* in tonsillar tissue and play an important role in the persistence of chronic infection [16–18]. Growth in biofilm provides a defense against host immune responses, can impede the access of macrophages, and can increase the tolerance to antibiotics [16,17]. Biofilm-associated antibiotic tolerance is a transient state in which normally susceptible bacteria enter homeostasis, which decreases sensitivity [19]. When these cells disperse and re-enter a plankton state, they regain normal antibiotic sensitivity [19]. In this study, we investigated the biofilm formation ability of *S. aureus* compared to biofilm production among strains and used it as a screening method to evaluate the clinical role of *S. aureus* in each study patient with RT. Samples from tonsillar crypts were obtained with punch biopsy needles, which is a novel technique for tonsillar sampling.

The objectives here were to evaluate the frequency and clinical importance of *S. aureus* colonization and biofilm production ability in patients with RT using patient samples from tonsillar crypts during tonsillectomy, and from the throat, nasal cavity, and armpits after tonsillectomy.

2. Results

The study group included 8 females and 8 males aged between 21 and 50 years, with a mean age of 29 years (±7.23). Seven patients lived in Riga, with the rest living in other cities in Latvia (Table 1).

During the past 3 years, 16 patients had from 2 to 7 recurrent episodes of tonsillitis. Three patients had a peritonsillar abscess in their medical history. Two patients had cryptolysis with radiofrequency, while 3 patients had cryotherapy before surgery. The last antibacterial treatment was received no earlier than 1 month before surgery. Three patients were hospitalized (1 patient in January 2018, 2 patients in 2017).

From tonsillar crypts the most commonly isolated bacteria was *S. aureus*, being the only microorganism in 6 patients and co-isolated with oral flora or with other potentially pathogenic microorganisms in 10 patients. Regarding *S. aureus*, 16 strains were isolated and tested for biofilm production, with 15/16 strains being biofilm producers. Furthermore, 1

of the strains was a strong biofilm producer, 5/16 strains were moderate, 9/16 were weak, and 1 strain of *S. aureus* did not produce a biofilm (Table 1).

Table 1. Patient characteristics and results of microbiological testing during and one year after tonsillectomy.

N	Sex	Age	Place of Residence	Microorganisms	*S. aureus* Biofilm Production				
					During TE	After TE			
					Tonsillar Crypts	Throat	Nasal Cavity	Armpits	
1	M	35	Bauska	***S. aureus*** + oral flora	1C weak	0	0	0	**Causative agent**
2	M	21	Saulkrasti	***S. aureus***	2C weak	0	2N moderate	0	**Predisposition to colonization**
3	F	27	Jurmala	***S. aureus*** + *Candida* spp.	3C weak	3T strong	0	0	**Predisposition to colonization**
4	F	25	Riga	***S. aureus*** + oral flora	4C weak	4T weak	0	0	**Part of patients' oral microbiome**
5	M	23	Babite	***S. aureus*** + oral flora	5C weak	5T weak	5N weak	5A weak	**Part of patients' oral microbiome**
6	M	50	Riga	***S. aureus***+ *Staphylococcus epidermidis*	6C moderate	0	0	0	**Causative agent**
7	F	35	Marupe	***S. aureus*** + oral flora	7C strong	0	0	0	**Causative agent**
8	M	24	Salaspils	***S. aureus***	8C moderate	0	0	0	**Causative agent**
9	F	33	Riga	***S. aureus*** + *Streptococcus pneumoniae* + *oral flora*	9C weak	0	0	0	**Causative agent**
10	F	31	Riga	***S. aureus*** + *Klebsiella pneumoniae* + *Candida* spp. + oral flora	10C weak	0	0	0	**Causative agent**
11	M	35	Riga	***S. aureus***	11C moderate	0	11N non—producer	0	**Predisposition to colonization**
12	M	24	Riga	***S. aureus***	12C weak	0	0	0	**Causative agent**
13	M	33	Saulkrasti	***S. aureus*** + *Neisseria subflava* + *Haemophilus influenzae* + *Streptococcus anginosus* + *Prevotella intermedia* + *oral flora*	13C weak	0	0	0	**Causative agent**
14	F	23	Ozolnieki	***S. aureus***	14C moderate	0	0	0	**Causative agent**
15	F	31	Rezekne	***S. aureus*** + oral flora + *Streptococcus agalactiae*	15C moderate	0	15N non—producer	0	**Predisposition to colonization**
16	F	28	Riga	***S. aureus***	16C non—producer	0	0	0	**Causative agent**

Note: M—male; F—female; TE—tonsillectomy; C—tonsillar crypts; T—throat; N—nasal cavity; A—armpits.

One year after tonsillectomy, 8 *S. aureus* strains were isolated from 6 out of 16 patients—from throat cultures in 3/16 patients, from nasal samples in 4/16 patients, and from armpit

samples in 1/16 patients. From throat samples, 1/3 was a strong biofilm producer and 2/3 strains of *S. aureus* were weak biofilm producers. From nasal samples, 1/4 was a moderate biofilm producer, 1/4 was a weak biofilm producer, and 2/4 strains of *S. aureus* did not produce a biofilm. From armpit samples, 1 strain of *S. aureus* did not produce biofilm. Only one patient had *S. aureus* in the throat, nasal, and armpit samples, and was a weak biofilm producer (Table 1).

S. aureus strains were isolated in 10 patients only during tonsillectomy; in these cases *S. aureus* was a causative agent of RT. Four patients had *S. aureus* in the throat or nasal cavity after tonsillectomy, but *S. aureus* isolates showed different degrees of biofilm formation. In these cases, patients had predisposition to *S. aureus* colonization. In 2 patients, *S. aureus* with the same biofilm production capacity was isolated from palatine tonsils during tonsillectomy and 1 year after tonsillectomy from the throat, nasal cavity, and axilla. In these cases, *S. aureus* was a part of the patients' microbiome (Table 1).

Figure 1 illustrates the biofilm formation of 11 *S. aureus* isolates in a 96-well flat-bottom microtiter plate. The crystal violet absorption is proportional to the adhesion cells and concentration of biofilm.

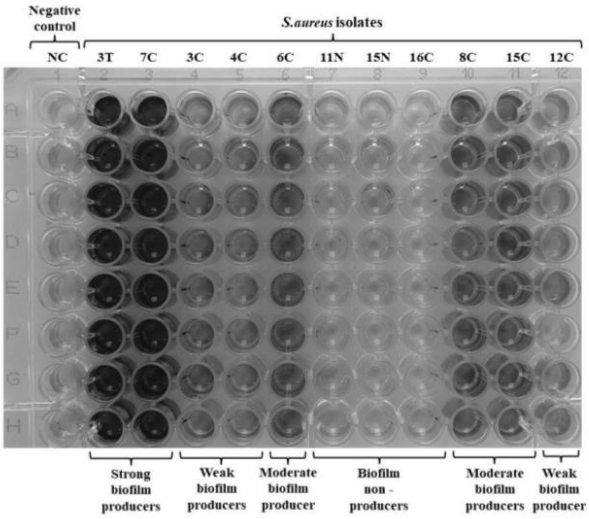

Figure 1. The biofilm formation of *S. aureus* isolates in a 96-well flat-bottom microtiter plate. The plate contained 11 strains and the negative control (NC) at 8 wells per strain. Staining was performed with crystal violet dye, differentiating strong (3T, 7C), moderate (6C, 8C, 15C), and weak biofilm producers (3C, 4C, 12C) and biofilm non-producers (11N, 15N, 16C). *S.aureus* isolate code – the number designates the patient and the letter - the carriage site (C—tonsillar crypts; T—throat; N—nasal cavity).

The crystal violet dye attached to the cells forming biofilms on microtiter plates was quantified. The optical density (OD) of the bacterial biofilm was measured at 570 nm wavelength with a microplate spectrophotometer. The OD values for each strain were expressed as a number. All mean OD values of isolated strain biofilms are summarized in Figure 2.

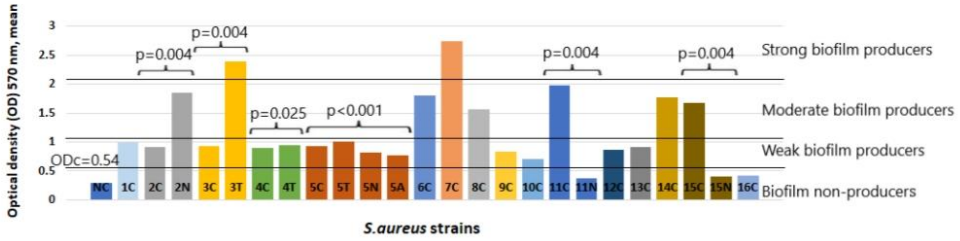

Figure 2. Biofilm production capability on microtiter plates of 24 isolates of *S. aureus*. Bars represent mean values of OD (measured at a wavelength of 570 nm). Trypticase soy broth with 1% glucose as a negative control (NC). The cut-off value (ODc) and biofilm production capacity levels are marked with horizontal lines. Differences between suspect *S. aureus* strain capability in biofilm formation were analyzed using Mann–Whitney U test for two-strain analysis and Kruskal-Wallis test for four-strain analysis, and are expressed as *p*-values. *S.aureus* strain code–the number designates the patient and the letter - the carriage site (C—tonsillar crypts; T—throat; N—nasal cavity; A—armpits).

Fourteen isolates were resistant to benzylpenicillin and ampicillin. One isolate was identified as MRSA, which was resistant to benzylpenicillin, ampicillin, cefoxitin, ceftriaxone, ampicillin–sulbactam, and amoxicillin with clavulanic acid, but intermediate resistance to ciprofloxacin. All isolates showed intermediate resistance to ciprofloxacin, while 1 isolate also showed intermediate resistance to clindamycin (Table 2).

Table 2. Antibiotic resistance among *S. aureus* strains isolated from patients with RT.

		Antibiotics										
		FOX	**CRO**	**P**	**AMP**	**AMS**	**AUG**	**CIP**	**AK**	**E**	**CD**	**C**
***S.aureus* strains**	1C	S	S	R	R	S	S	I	S	S	S	S
	2C	S	S	S	S	S	S	I	S	S	S	S
	2N	S	S	R	R	S	S	I	S	S	S	S
	3C	S	S	R	R	S	S	I	S	S	S	S
	3T	S	S	S	S	S	S	I	S	S	S	S
	4C	R	R	R	R	R	R	I	S	S	S	S
	4T	S	S	R	R	S	S	I	S	S	S	S
	5C	S	S	R	R	S	S	I	S	S	S	S
	5T	S	S	S	S	S	S	I	S	S	S	S
	5N	S	S	S	S	S	S	I	S	S	S	S
	5A	S	S	S	S	S	S	I	S	S	S	S
	6C	S	S	R	R	S	S	I	S	S	S	S
	7C	S	S	R	R	S	S	I	S	S	S	S
	8C	S	S	R	R	S	S	I	S	S	S	S
	9C	S	S	R	R	S	S	I	S	S	S	S
	10C	S	S	R	R	S	S	I	S	S	S	S
	11C	S	S	S	S	S	S	I	S	S	S	S
	11N	S	S	S	S	S	S	I	S	S	S	S
	12C	S	S	R	R	S	S	I	S	S	S	S
	13C	S	S	R	R	S	S	I	S	S	I	S
	14C	S	S	S	S	S	S	I	S	S	S	S
	15C	S	S	R	R	S	S	I	S	S	S	S
	15N	S	S	R	R	S	S	I	S	S	S	S
	16C	S	S	S	S	S	S	I	S	S	S	S

FOX, cefoxitin; CRO, ceftriaxone; P, benzylpenicillin; AMP, ampicillin; AMS, ampicillin–sulbactam; AUG, amoxicillin–clavulanic acid; CIP, ciprofloxacin; AK, amikacin; E, erythromycin; CD, clindamycin; C, chloramphenicol. S, sensitive; R, resistant; I, intermediate. *S.aureus* strain code–the number designates the patient and the letter-the carriage site (C—tonsillar crypts; T—throat; N—nasal cavity; A—armpits). Coloring: *S.aureus* strain code colors correspond to the optical density bars of the same isolates in Figure 2; greyish–antibiotics; rosy–resistant; yellowish–intermediate.

3. Discussion

The anterior nares represent the dominant ecological niche, while other sites that can be colonized include the axilla, perineum, and pharynx [4]. Elimination of the nasal carriage by topical antibiotics generally leads to loss of carriage in these areas [20]. *S. aureus* readily recolonizes the nose, throat, and other sites within several months after antibiotic treatment [21,22]. In *S. aureus* carriers, infection rates are higher than in non-carriers, and patients are usually infected by the same strains with which they are colonized [17]. In our study, infected palatine tonsils were removed surgically, and the presence of *S. aureus* in the pharynx, axilla, and nares was assessed 1 year after tonsillectomy.

There are host and bacterial factors that can influence the carriage of *S. aureus*. The main predisposing factors to staphylococcal infection development include age, the presence of chronic diseases or immunodeficiency, genetics, direct contact with healthcare settings, and hospitalization [2]. Patients enrolled in the study were young individuals (mean age 29 years) without high carriage rate host factors such as HIV infection, insulin-dependent diabetes, continuous ambulatory peritoneal dialysis and hemodialysis, or intravenous drug use [4,23–25]. The mean age of our study patients was consistent with the data from other studies, in which tonsillectomy patients were 28 years old [26,27].

We are aware of the disadvantages of routine surface sampling of tonsils. Tonsillar core and tonsillar crypt samples are more favorable for culturing [6,8]. Crypts are narrow passages that penetrate the palatine tonsils. It is not possible to obtain the contents of crypts with a cotton swab. In our study, samples from the palatine tonsils were obtained with a punch biopsy needle, which is suitable for the width and depth of the crypts to obtain optimal size samples [28].

Biofilm formation is one of the bacterial factors that is distinctive for the adhesive phenotype of bacteria [4]. Bacteria in biofilm state present differential metabolic and physiological functions, often rendering them more virulent and resistant to antibiotics [29]. Neopane and co-authors (2018) showed that biofilm-producing *S. aureus* isolated from wounds was more resistant to various antimicrobials than the biofilm non-producers [30].

A broad range of assays for biofilm quantification in microtiter plates have been described [31,32]. We used the crystal violet assay adapted from Stepanovic et al. (2007) because it is reliable, cost-effective, straightforward, and is commonly used for the quantification of biofilm production by staphylococci [33]. It is also important that this method can be easily performed by other investigators. Because both living and dead cells, as well as the matrix, are stained by the crystal violet dye, this method is poorly suited for differentiation between living and dead cells, and susceptibility testing of biofilms cannot be performed [31]. The drawbacks of the method do not affect the research question being investigated in our study. However, other methods such as flow cell systems would increase data reliability and would allow more detailed biofilm investigations, specifically in dynamic conditions.

In our study, emphasis was placed on the biofilm formation ability of *S. aureus* isolated strains. *S. aureus* strains isolated from the tonsillar crypts, as compared to isolates collected from other body sites had greater capacity to produce biofilms. Biofilm-producing *S. aureus* strains were mostly isolated from tonsillar crypts and were susceptible to the majority of tested antibiotics. Only one isolate was identified as MRSA, which showed a wider spectrum of resistance and was a weak biofilm producer. The isolate was obtained from a 25-year-old female without co-morbidities, with 5 episodes of tonsillitis per year for the last 3 years. After tonsillectomy the MRSA strain was eradicated. MRSA strains were detected in RT patients in other studies also. In the study conducted by Katkowska et al., the MRSA strain was isolated from the tonsils in one out of 118 adult patients and in two out of 73 children qualified for tonsillectomy in Poland [7,12]. The role of biofilm formation and the antimicrobial resistance of MRSA and methicillin-susceptible *S. aureus* (MSSA) are unclear [34]. The environmental factors (temperature, pH, glucose level, type of media, and others) influence bacterial biofilm production. Therefore, these factors should be accounted for in biofilm research. To compare the results from different studies, one should use

similar or even the same biofilm method and environmental factors [35]. Our study results showed that in 10 patients tonsillectomy resulted in no growth of *S. aureus* strains a year later; therefore, tonsillectomy could prevent bacterial colonization within a one year period. However, to prove such phenomena, this should be investigated in bigger cohorts.

Our study underlines the immense importance of studying *S. aureus* colonization to understand the pathology of staphylococcal disease. Current efforts to interrupt carriage rely on the use of antibiotics, but the development of efficacious antibiofilm *S. aureus* therapies is a new and necessary perspective.

4. Strengths and Limitations

The study provides a one year follow-up period for surgically treated patients with recurrent tonsillitis. Furthermore, this study provides evaluations of the outcomes and the effects of tonsillectomy on the results of microbiological testing.

However, several limitations should be mentioned. Firstly, the small number of cases observed during the study period could cause bias. Further studies, including those with a larger study group, a control group, an increased bacterial spectrum with biofilm formation, antibacterial susceptibility, and bacterial genotyping, would be necessary to draw reliable conclusions regarding tonsillitis.

5. Materials and Methods

A case series study was performed in Pauls Stradins Clinical University Hospital in Riga, Latvia. The study lasted from 2018 to 2020. The study was approved by the local ethics committee of Riga Stradins University (document No. 49/30 November 2017). Written consent was obtained from every patient. The exclusion criterion was recent (less than a month ago) antibacterial treatment. A control group was not applied. Patient medical history information was gathered from patients and via their medical records by a general practitioner and anesthesiologist before elective tonsillectomy.

5.1. Isolation of Microorganisms and Antibacterial Susceptibility Testing

Samples from tonsillar crypts were obtained with a punch biopsy needle from 16 adults undergoing tonsillectomy for RT. For research purposes, the punch biopsy needle was designed with a prolonged and curved handle and a circular blade for tonsillar crypt biopsy (patent number: LVP2020000055) [28]. Swab samples from the throat, nasal cavity, and armpit were obtained a year later to assess *S. aureus* carriage. Materials were transported with universal transport media (AMIES) at room temperature within 24–48 h.

The obtained materials were inoculated in blood agar, chocolate agar (with test discs loaded with oleandomycin in a CO_2-enriched atmosphere), CAN agar (with optochin disk test), Brucella blood agar, mannitol salt agar, MacConkey agar, and Saburo agar plates and incubated under aerobic conditions for 48 h at 36 ± 2 °C temperature. The isolated microorganisms underwent the macro- and microscopic evaluations. The isolated bacteria were identified by Gram staining using a VITEK-2 Compact device (bioMériux, Marcy l'Étoile, France).

Disk diffusion antimicrobial susceptibility tests were performed and the results were evaluated according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), *Clinical Breakpoints and Dosing of Antibiotics* (Version 10.0, January 2020) [36].

5.2. Biofilm Growth Using Cristal Violet Assay

A crystal violet assay adapted from Stepanovic et al. (2007) was used for the in vitro cultivation and quantification of bacterial biofilms [33]. Isolated *S. aureus* strains were suspended in trypticase soy broth (TSB) supplemented with an additional 1% glucose for incubation at 37 °C for 16–18 h. Inoculated broths were diluted with un-inoculated TSB at a ratio of 1:100. Then, 150 µL of diluted suspension was transferred using a multi-channel pipette into a sterile 96-well plate (Thermo Scientific™ Nunc MicroWell 96-Well

Microplates (flat-bottomed), Thermo Fisher Scientific, Roskilde, Denmark). Each plate contained 11 strains and the negative control (sterile broth) at 8 wells per strain, and each experiment was performed in triplicate. The inoculated plates were cultivated aerobically at 37 °C for 48 h. After incubation, all wells were emptied by throwing out the liquid in a clinical waste bag without using a pipette. Each well was rinsed 3 times with 250 µL 0.9% saline. After washing, staining was performed by adding 150 µL of 0.1% crystal violet per well. After 15 minutes, the color was removed by throwing it out and each well was washed 3 times with 250 µL distilled water. At the end, 150 µL of 96% ethanol was added to each well. Afterwards, the optical density (OD) of wells was measured at 570 nm wavelength with a microplate spectrophotometer (Tecan Infinite F50, Mannedorf, Switzerland, with Magellan™ reader control and data analysis software V 6.6) [37].

5.3. Biofilm Calculation

The OD values for each strain were averaged and expressed as a number. The cut-off value (ODc) was defined as three standard deviations above the mean OD of the negative control and was separately calculated for each experiment. Strains were divided as follows: OD $\leq$ ODc = no biofilm producer, ODc < OD $\leq$ 2 $\times$ ODc = weak biofilm producer, 2 $\times$ ODc < OD $\leq$ 4 $\times$ ODc = moderate biofilm producer, 4 $\times$ ODc < OD = strong biofilm producer [25].

5.4. Data Analysis

Data analysis was performed using SPSS software (IBM SPSS Statistics version 26) and Microsoft Excel 10.5.5. Assessment of Outcomes

Outcomes were categorized as:

(1) A causative agent of RT—*S. aureus* strains were isolated only in tonsillar crypts, while no *S. aureus* was recovered from any site after tonsillectomy;
(2) Predisposition to colonization—*S. aureus* strains were isolated during and also after tonsillectomy, but *S. aureus* strains from one individual showed different phenotypes in their biofilm formation profiles;
(3) Parts of patients' oral microbiomes—*S. aureus* strains were isolated during and also after tonsillectomy, but *S. aureus* strains showed no phenotypical changes in biofilm formation.

6. Conclusions

S. aureus is a common cause of recurrent tonsillitis in our cohort population. Tonsillectomy results in less frequent isolation of *S. aureus* strains. Isolation of *S. aureus* from multiple sites and the determination of the biofilm production capacity of isolated strains is a way of distinguishing carriers of *S. aureus*.

7. Patents

Klagisa, R.; Kroica, J.; Kise, L. Punch Biopsy Needle. Patent No: LVP2020000055. In *Izgudrojumi, Preču Zīmes un Dizain-paraugi*. Patent Office of the Republic of Latvia, Riga, Latvia, 2021; Volume 5, pp. 315.

Author Contributions: Conceptualization, J.K., K.R., and L.K.; methodology, J.K. and K.R.; acquisition of clinical samples, R.K.; biofilm and antibacterial susceptibility testing, R.K. and R.B.; investigation, K.R. and R.K.; resources, R.K.; data curation, R.K., R.B., and K.R.; writing—original draft preparation, R.K.; writing—review and editing, K.R.; visualization, K.R. and R.K.; supervision, J.K. and L.K.; project administration, R.B.; funding acquisition, J.K. and R.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Riga Stradins University (document No. 49/30.11.2017.).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets generated are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

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5. pielikums

Recidivējoša tonsilīta un peritonsilārā abscesa pacientu izdalīto mikroorganismu dažādība

1. tabula

Mikroorganismi, kas izolēti no 99 RT pacientu aukslēju mandeļu kriptām

Izolātu kombinācijas	**Skaits (n = 99)**
Normālā orālā mikrobiota	39
Staphylococcus aureus	14
Staphylococcus aureus + normālā orālā mikrobiota	7
Staphylococcus aureus + *Candida* spp.	1
Staphylococcus aureus + *Lactobacillus paracasei*	1
Staphylococcus aureus + *Streptococcus parasanguinis*	1
Staphylococcus aureus + *Staphylococcus epidermidis*	1
Staphylococcus aureus + *Haemophilus influenzae*	1
Staphylococcus aureus + *Klebsiella pneumoniae*	2
Staphylococcus aureus + *Klebsiella oxytoca*	1
Staphylococcus aureus + *Streptococcus agalactiae* + normālā orālā mikrobiota	1
Staphylococcus aureus + *Klebsiella pneumoniae* + *Candida* spp. + normālā orālā mikrobiota	1
Staphylococcus aureus + *Streptococcus pneumoniae* + normālā orālā mikrobiota	1
Staphylococcus aureus + *Prevotella intermedius* + *Streptococcus oralis* + normālā orālā mikrobiota	1
Staphylococcus aureus + *Streptococcus anginosus* + *Neisseria subflava* + *Haemophilus influenzae* + *Prevotella intermedius* + normālā orālā mikrobiota	1
Staphylococcus pseudintermedius	1
Staphylococcus capitis + *Aggregatibacter aphrophilus*	1
Streptococcus parasanguinis	1
Streptococcus anginosus	1
Streptococcus anginosus + normālā orālā mikrobiota	1
Streptococcus anginosus + *Candida* spp.	1
Streptococcus pyogenes + *Streptococcus pneumoniae*	1
Streptococcus pyogenes + *Morganella morganii*	1
Streptococcus oralis + normālā orālā mikrobiota	1
Streptococcus oralis + *Streptococcus mitis* + normālā orālā mikrobiota	1
Escherichia coli	2
Escherichia coli + *Staphylococcus epidermidis*	1
Klebsiella pneumoniae	5
Klebsiella pneumoniae + *Streptococcus pyogenes*	1
Klebsiella pneumoniae + *Candida* spp.	1
Klebsiella pneumoniae + *Streptococcus pyogenes* + *Candida* spp. + normālā orālā mikrobiota	1
Serratia rubidea	1
Burkholderia gladioli	1
Pseudomonas aeruginosa	1
Candida spp.	2
Candida spp. + normālā orālā mikrobiota	1

2. tabula

Mikroorganismi, kas izolēti no 29 PTA pacientu aukslēju mandeļu kriptām

Izolātu kombinācijas	Skaits (n = 29)
Normālā orālā mikrobiota	5
Staphylococcus aureus	1
Staphylococcus aureus + normālā orālā mikrobiota	1
Staphylococcus aureus + *Staphylococcus capitis*	1
Staphylococcus aureus + *Staphylococcus epidermidis*	1
Staphylococcus aureus + *Candida* spp.	1
Staphylococcus aureus + *Escherichia coli* + *Candida* spp.	1
Staphylococcus aureus + *Streptococcus oralis* + *Streptococcus mitis* + *Streptococcus sinensis* + normālā orālā mikrobiota	1
Staphylococcus epidermidis	1
Klebsiella pneumoniae	2
Klebsiella pneumoniae + *Candida* spp.	2
Klebsiella oxytoca + *Candida* spp. + normālā orālā mikrobiota	1
Acinetobacter ewoffi + normālā orālā mikrobiota	1
Bacillus cereus + *Prevotella intermedius*	1
Candida spp.	5
Candida spp. + normālā orālā mikrobiota	3
Candida spp. + *Actinomyces*	1

Rīgas Stradiņa universitātes Pētījumu ētikas komitejas atļauja

Veidlapa Nr. E-9 (2)

RSU ĒTIKAS KOMITEJAS LĒMUMS NR. 49 / 30.11.2017.

Rīga, Dzirciema iela 16, LV-1007
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4.	Asoc.prof. Voldemārs Arnis	Dr.biol.	rehabilitologs
5.	Profesore Regīna Kleina	Dr.med.	patalogs
6.	Profesors Guntars Pupelis	Dr.med.	ķirurgs
7.	Asoc.prof. Viesturs Liguts	Dr.med.	toksikologs
8.	Docente Iveta Jankovska	Dr.med.	
9.	Docents Kristaps Circenis	Dr.med.	

Pieteikuma iesniedzējs: **Dr. Renāta Klagiša**
Medicīnas fakultāte, Doktorantūra

Pētījuma nosaukums: "Hroniska tonsilīta agrīnas diagnostikas iespējas."

Iesniegšanas datums: 30.11.2017.

Pētījuma protokols: Izskatot augstāk minētā pētījuma pieteikuma materiālus (protokolu) ir redzams, ka pētījuma mērķis tiek sasniegts veicot ar pacientiem, bez kāda apdraudējuma veselībai, drošībai un dzīvībai, objektīvu izmeklēšanu, anamnēzes datu ievākšanu un operācijas materiālu izvērtēšanu, iegūto datu apstrādi un analīzi, kā arī izsakot priekšlikumus. Personu (pacientu, dalībnieku) fizisko datu aizsardzība, informēta brīvprātīga piedalīšanās un konfidencialitāte ir ievērota un nodrošināta. Līdz ar to pieteikums atbilst pētījuma ētikas prasībām.

Izskaidrošanas formulārs: ir

Piekrišana piedalīties pētījumā: ir

Komitejas lēmums: **piekrist** pētījumam

Komitejas priekšsēdētājs Olafs Brūvers Tituls: Dr. miss., prof.

Paraksts

RĪGAS STRADIŅA UNIVERSITĀTE ĒTIKAS KOMITEJA

Ētikas komitejas sēdes datums: 30.11.2017.

Veidlapa Nr. E-9(3)
APSTIPRINĀTA
ar Rīgas Stradiņa universitātes rektora
2018. gada 26. septembra rīkojumu Nr. 5-1/238/2018

Rīgas Stradiņa universitātes
Pētījumu ētikas komitejas
LĒMUMS
Rīgā

10.09.2020. Nr.6-1/09/ 22

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2	Asoc.prof. Santa Purviņa	Dr.med.	farmakologs
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4	Profesore Regīna Kleina	Dr.med.	patalogs
6	Asoc.prof. Viesturs Liguts	Dr.med.	toksikologs
7	Docente Iveta Jankovska	Dr.med.	ortodonts
8	Docents Kristaps Circenis	Dr.med.	docētājs

Pieteikuma iesniedzējs/i: **Renāta Klagiša**
Doktorantūra

Pētījuma / pētnieciskā darba nosaukums: "Biofilmas veidošanās spēja un tās saistība ar antibakteriālo jutību hroniska tonsilīta pacientiem".

Iesniegšanas datums: 02.02. 2020.

Pētījuma protokols:
Izskatot augstāk minētā pētījuma pieteikuma materiālus (protokolu) ir redzams, ka pētījuma mērķis tiek sasniegts veicot pacientu medicīniskās dokumentācijas (slimības vēstures, vai klīniskā gadījuma, citi izmeklējumu dati) izpēti, iegūto datu apstrādi un analīzi, kā arī izsakot priekšlikumus. Personu (pacientu, dalībnieku) datu izmantošana, glabāšana, aizsardzība, anonimitāte un konfidencialitāte ir ievērota un nodrošināta. Līdz ar to pieteikums atbilst pētījuma ētikas prasībām.

Komitejas lēmums: **piekrist** pētījumam

Komitejas priekšsēdētājs Olafs Brūvers Tituls: Dr. miss., prof.

Paraksts

RĪGAS STRADIŅA UNIVERSITĀTE ĒTIKAS KOMITEJA

I.Bēniņa
67061596

7. pielikums

Anketa pacienta datu uzskaitei pētījuma ietvaros

Nr.	Vecums	Dzimums	Tonsilīta recidīvu biežums	Lietotā antibakteriālā terapija	Iepriekš lietotā ārstēšana, efekts	Parauga paņemšanas veids, vieta, veikšanas datums
1.						

8. pielikums

Piekrišanas veidlapa dalībai pētījumā

Pētījums: Biofilmu nozīme recidivējoša tonsilīta un paratonsilārā abscesa patoģenēzē un klīniskajā norisē

Pētījuma ilgums
Jūsu piedalīšanās pētījumā sastāvēs no vienreizējas apskates.

Procedūras
Ja Jūs piekrītat piedalīties pētījumā,
- Ar Jums runās ārsts, lai iegūtu nepieciešamo personas un klīnisko informāciju;
- Jūs izmeklēs ārsts;
- Laboratoriski tiks analizēts Jūsu operācijas materiāls.

Jūs varat tikt iekļauts pētījumā, ja esat hospitalizēts ar aukslēju mandeļu slimību, kuras dēļ tiek plānota ķirurģiska ārstēšana – tonsilektomija.

Risks un neērtības (diskomforts)
Pacientiem, kuri piedalīsies pētījumā, nav paredzamas diskomforta situācijas.

Ieguvumi
- Operācijas materiāla mikrobioloģiska analīze.
- Zinātniski pamatoti ieteikumi recidivējoša tonsilīta agrīnai diagnostikai pēc pētījuma pabeigšanas.

Konfidencialitāte
Pētījumā iegūtie dati tiks glabāti konfidenciāli un netiks doti nevienam, kas nav iesaistīts pētījumā. Pētījuma informācija tiks glabāta saskaņā ar likumu. Visiem pētījumā iesaistītiem darbiniekiem ir prasība neatklāt Jūsu personību. Informācija par Jums netiks nodota citām ar pētījumu nesaistītām personām bez Jūsu atļaujas.

Apliecinājums, ka ņemtais materiāls netiks izmantots citiem nolūkiem, kā norādīts pētījumā
Pētījuma laikā ņemtais materiāls tiks izmantots tikai tiem nolūkiem, kas nepieciešami projekta ietvaros noritošai izpētei.

Tiesības atteikties vai pārtraukt piedalīšanos pētījumā
Jūsu piedalīšanās pētījumā ir pilnīgi brīvprātīga. Ja Jūs esat piekritis/-usi pētījumam, Jums ir tiesības jebkurā brīdī atteikties no dalības pētījumā. Jūsu lēmums piedalīties vai atteikties no dalības pētījumā neietekmēs Jūsu medicīniskās aprūpes kvalitāti vai attiecības ar Jūsu ārstu.

Jautājumi vai bažas
Jums vajadzētu uzdot jautājumus ārstam par jebkuru neskaidrību, kas saistīta ar šo pētījumu. Jums ir tiesības uzdot jautājumus par pētījuma norisi dr. R. Klagišai, prof. L. Ķīsei, prof. J. Kroičai.

Institucionāls apstiprinājums
Šo projektu ir apstiprinājusi Rīgas Stradiņa universitāte un Paula Stradiņa klīniskā universitātes slimnīca.

Es ar savu parakstu apliecinu, ka:

1. **Esmu saņēmis/-usi un iepazinies/-usies ar rakstisku informāciju par pētnieciskā projekta "Biofilmu nozīme recidivējoša tonsilīta un paratonsilārā abscesa patoģenēzē un klīniskajā norisē" (turpmāk – Pētījums) mērķi un saturu. Uz visiem jautājumiem esmu saņēmis/-usi izsmeļošas atbildes.**
2. **Esmu saņēmis/-usi un iepazinies/-usies ar informāciju par manu personas datu apstrādes tiesisko pamatu, mērķi, apjomu, glabāšanas ilgumu un iznīcināšanu, kā arī esošo un iespējamiem personas datu saņēmējiem.**
3. **Esmu informēts/-a, ka jebkura mani identificējoša informācija būs konfidenciāla un mana personas datu apstrāde tiks veikta atbilstoši labas prakses principiem un saskaņā ar spēkā esošo normatīvo aktu prasībām.**
4. **Piekrītu Pētījuma diagnostikas pakalpojumu rezultātā iegūto personas datu apstrādei un uzglabāšanai, kas nepieciešama Pētījuma mērķa sasniegšanai, t. sk., ka mani personas dati tiek nodoti sertificētiem medicīnas speciālistiem zinātnes un pētniecības nolūkiem.**
5. **Esmu informēts/-a, ka jebkurā brīdī bez paskaidrojumiem varu pārtraukt piedalīšanos Pētījumā un tas neietekmēs manu turpmāko ārstēšanu.**
6. **Esmu informēts/-a, ka man ir tiesības jebkurā brīdī labot, papildināt vai pārtraukt manu personas datu apstrādi vai jautāt par personas datu iznīcināšanu, kā arī man ir tiesības iegūt visu informāciju, kas ir savākta par maniem personas datiem.**

7. **Personas datu apstrāde pēc Pētījuma beigām (vajadzīgo atzīmēt):**

	Es PIEKRĪTU,		**Es NEPIEKRĪTU,**
ka Pētījuma ietvaros savāktie un/vai iegūtie personas dati turpmāk glabāsies arī RSU Bioloģijas un mikrobioloģijas katedras laboratorijā un var tikt izmantoti ar mandeļu slimībām saistītos pētījumos – bez ierobežojuma;			

(pacienta paraksts un atšifrējums) Datums

Pētījumā iesaistītais ārsts:
Apstiprinu, ka esmu informējis pacientu par šo pētījumu
(paraksts un atšifrējums / spiedogs):

_____ Datums

Piezīme. Parakstītās kopijas pa vienai jānodod projekta vadītājam, pacientam un jāievieto slimības vēsturē.