ORIGINAL ARTICLES

ASSESSMENT OF ANTIBIOTIC RESISTANCE IN *STREPTOCOCCUS* ISOLATES CAUSING INFECTION OF THE ORAL CAVITY IN COVID-19 RECOVERED PATIENTS

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Abstract

Objective: This study evaluates the load of *Streptococcus* species and assesses their antibiotic resistance in COVID-19-recovered patients (Group A) and healthy patients (who never suffered from COVID-19, Group B) attending the out-patient department in tertiary care dental hospital in Bhubaneswar, Odisha.

Methodology: Unstimulated saliva samples were collected from 25 patients of each group and were screened for *Streptococcus* species. Further species-level identification was done using routine microbiological, biochemical, antigen-detection kits, and PCR techniques. The antibiotic sensitivity test was carried out using the Kirby-Bauers disk diffusion test.

Results: Five different species of *Streptococcus* were isolated. In both groups, *Streptococcus mutans* isolates were more in number, followed *by S. pyogenes*. Our study also recorded that the *Streptococcus* strains isolated from COVID-19-recovered patients were resistant to more antibiotics than those isolated from non-COVID patients.

Conclusion: In conclusion, there has been a significant rise in the MDR strains of *Streptococcus* species in India and globally. In our study, COVID-19-recovered patients had more *Streptococcus* species isolated from their oral cavity than strains isolated from the healthy controls. Hence, dental hospitals and clinics can implement modified safety regulations and antibiotic policies to reduce infections and antibiotic resistance problems.

Key words: *Covid 19; Streptococcus species; Antibiotic resistance; Oral infections; Oral Microbiome.*

Introduction

The SARS-CoV-2 infection, originally started in Wuhan, China, in December 2019, has resulted in several post-COVID-19 clinical complications. This pandemic also warned of giving rise to several opportunistic co-infections among COVID-19-infected individuals[1]. The coronavirus mainly affected the oropharyngeal region of COVID-19 patients, adversely affecting this region and disturbing the oral microbiota. The oral cavity is a reservoir of 700 species of normal flora and the central portal of entry of microbial pathogens. *Streptococcus* species are the most prevalent one around the oropharyngeal region in the human body and can invade easily [2,3]. Though *Streptococcus* is one of the oldest inhabiting bacteria in human beings, these pathogens invade and proliferate, which may result in dental caries and other periodontal conditions [4,5]. As a result, antibiotics were widely provided to COVID-19 patients even though antibiotics are useless against viruses such as COVID-19. Almost 80% of individuals hospitalized with COVID-19 received antibiotics in some manner [6,7]. The escalation of antibiotic resistance in *Streptococci* has been associated with several mechanisms, including efflux pumps and antimicrobial target modifications. Antibiotic resistance emerges from previously sensitive populations of *Streptococci* due to horizontal gene transfer or chromosomal point mutations caused by antimicrobial overuse. *Streptococci* strains are also known to produce biofilms. Increased antibiotic resistance of *Streptococci* biofilms promotes persistent infection, accounting for approximately 80% of human microbial infections [8,9]. Therefore, there have been concerns that increased antibiotic use (both prescribed and unprescribed) to treat secondary infections associated with COVID-19 has led to antibiotic resistance among these normal flora and incoming pathogenic bacteria; however, direct evidence has been lacking [10,11]. Hence, assessing a load of *Streptococcal* infection and their response to antibiotic treatments in both COVID-19-recovered patients and non-COVID patients (patients who never tested positive) becomes essential. While the influence of COVID-19 pandemic on drug-resistant bacteria is yet unknown, it is apparent that there will be a shifting set of global threats to antibiotic resistance [12-16].

Numerous studies have been conducted on *Streptococcal* infections and their antibiotic resistance among various health groups. However, this study will be the first to check the occurrence of *Streptococcal* infections among COVID-19-recovered patients and its oral manifestation among them. Further, this study intends to check the load of *Streptococcus* species and assess their level of antibiotic resistance from COVID-19-recovered patients and healthy patients (who never

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suffered from COVID-19) attending the OPD in tertiary care dental hospital in Bhubaneswar, Odisha.

Materials and Methods

Study design

A hospital-based cross-sectional study was conducted among COVID-19 recovered (Group A) patients and non-COVID-19 patients (Group B, never diagnosed with COVID-19) visiting OPD of a tertiary care dental hospital in Eastern India, from March 2023 for Oral health conditions. The demographic and clinical data of the patients were documented in Excel sheets.

Ethical Permission

This study was carried out after the approval of the Institute Ethical Committee of the Dental Sciences via letter no. IEC-IDS/IDS.SOA/2023/I-41 dated 10th October 2023.

Sampling Method

A simple random probability sampling technique was used for the regular patients visiting the OPD of a tertiary care dental hospital in Bhubaneswar, Odisha. Fifty saliva samples (25 from COVID-recovered patients and 25 from non-COVID patients) were collected from the periodontal pocket and tested for antibiotic sensitivity.

Inclusion Criteria

i. COVID-19-recovered patients diagnosed with *Streptococcus* infections
ii. Non-covid-19 patients diagnosed with *Streptococcus* infections

Exclusion Criteria

i. Patients with multiple oral infections

Sample Collection

2 ml of unstimulated saliva was collected from the selected patients and immediately transferred to the sterile holding medium. Saliva samples mixed with holding medium were kept at room temperature, and then 2 ml of fresh 2 ml sample mixture was mixed with 8 ml of sterile normal saline and shaken to form a homogenous mixture. All samples were mixed by vigorous shaking and serially diluted.

Processing of Samples for Microbiological and Biochemical Investigations

All the samples were taken to the Central Research Laboratory, IDS, for further processing. Bacteria were incubated overnight at 37°C on blood agar plates for 24 h. The colonies formed on the plate were subjected to gram staining, routine microbiological and biochemical test for identification. Later, *Streptococci* species was confirmed using molecular biology method[17].

Microbiological Identification

The initial procedure involves conducting a Gram stain on the specimen. *Streptococci* are a type of Gram-positive cocci that exhibit a purple colouration when observed via a microscope. In addition, *Streptococci* are frequently categorized according to their capacity to induce lysis of erythrocytes on blood agar plates. This phenomenon is called hemolysis and can be classified into three distinct types: Alpha-hemolysis, Beta-hemolysis, and Gamma-hemolysis. *Streptococci* have a catalase-negative characteristic, indicating their lack of enzyme catalase production. Adding a small quantity of hydrogen peroxide to a colony on a slide results in the absence of bubble formation, indicating that the organism is catalase-negative. For sugar fermentation tests, a pure culture's inoculum is aseptically transferred to a sterile tube with phenol red sucrose broth. The inoculation tube is incubated at 35-37°C for 24 hours, after which the results are assessed. A positive test is characterized by a transition in hue from red to yellow, which signifies a shift in pH toward acidity [17, 18].

Antigenic Detection of Streptococcal Groups

The *Streptococca*l groups A, B, C, D, F, G, and H were efficiently identified using the process of latex agglutination, utilizing the Streptococcal Grouping Kit (Himedia, Mumbai). The primary objective of this quick latex agglutination test was to provide straight forward and expeditious outcomes for the detection and classification of *Streptococcal* groups A, B, C, D, F, G and H. The test employs latex particles coated with antibodies specific to a particular group. These antibodies clumped together when they got exposed to homologous antigens[18].

PCR Identification

The universal PCR performed with 50µl DNA sample was extracted from dental carries in a total reaction volume of 25 µl, consisting of 12 µl of master mix, 1µl of forward primer, 1 µl of reverse primer,3µl of DNA sample, and 8 µl of molecular grade water. PCR was carried out with a semiquantitative thermal cycler under the following conditions: initial denaturation 95 °C for 10 min, denaturation 94 °C for 2 min, annealing 55-60 °C for 30 sec, and then extension 72 °C for 45 sec and then repeat the three cycles 30 times and then final extension 72 °C 10 min. To detect the PCR product, 10 µl of amplified DNA was run on a 1% agarose gel with dye and ethidium bromide and visualized under UV light. DNA was isolated using a DNA isolation Kit (HiMedia, Mumbai) through agarose gel electrophoresis. 16S rDNA amplified with universal primer pairs 27F, 1525R (A); 27F, 1492R (B) and 530F, 1525R (C). Amplified genes were identified by comparison with 16S rRNA databases [19].

Antibiotic Sensitivity Test

All identified strains were subjected to antibiotic sensitivity tests by Kirby-Bauer's using a 4 mm thick blood agar/Muller Hinton agar. An aliquot of 0.1 mL of the exponentially growing culture was spread on agar for lawn development at 37°C in an incubator. Further, on the lawn-agar of each plate, eight antibiotic discs

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(HiMedia, Mumbai) were placed at equal distances from one another. Plates were incubated for 18 h at 37°C. The zone of inhibition around each antibiotic disk was measured and compared to the standard antibiotic susceptibility test chart of Clinical Laboratory Standard Institute guidelines[20].

The results were similar to *S. mutans* and *S. salivarius.* Further, *S. mitis* is gram-positive cocci with gamma-hemolytic colonies and is catalase-negative. Lastly, *S. pyogenes* was gram positive with beta-hemolytic colonies (Table 1).

Table 1. Gram staining, hemolytic, sugar fermentation, and antigenic detection test results of isolated different *Streptococcus* species

Isolated bacteria	Gram Stain	Colony characters on Blood Agar	Colony characters on Nutrient agar	Hemolysis	Sugar fermentation test		Antigenic Detection *Streptococcal* Groups
					Positive	Negative	
S. sanguis	+ve cocci	Glossy, round, translucent colonies	Glossy, round, translucent colonies	α	Lac, Raf, Tre	Man, Sor, VP	Group H
S. mitis	+ ve cocci	Round, grey, elevated, small colonies	Round, colorless/non-pigmented convex colonies	α	Lac	Man, Raf, Sor, Tre, VP	Viridans
S. mutans	+ ve cocci	Round, grey, elevated, small colonies	Round, greyish-white, elevated, small colonies	α	Man, Lac, Raf, Sor, Tre, VP		Group A
S. salivarius	+ ve cocci	Round, convex, white mucoid colonies	Round, convex, white butyrous colonies	α	Lac, Raf, Tre	Man, Sor, VP	Viridans
S. pyogenes	+ ve cocci	Round, Pinpoint, opaque Light yellow/off-white, matt colonies	Round, Pinpoint, opaque Light yellow/off-white, matt colonies	β	Lac, Tre	Man, Raf, Sor, VP	Group A

Note: Man: Mannitol, Lac: Lactose; Raf: Raffinose; Sor: Sorbitol; Tre: Trehalose, VP: Voges-Prausker;

Results

Fifty saliva samples (25 from COVID-recovered patients and 25 from non-COVID patients) were collected from the periodontal pocket to isolate *Streptococcus* species and tested for antibiotic sensitivity. The patients' COVID status was confirmed while obtaining the patients' consent. The patients were not segregated based on gender or age as the number of The COVID-19 recovered patients was less, and they were primarily male The isolated bacteria were differentiated based on gram staining results, hemolysis pattern, colony characteristics on blood agar, and nutrient agar. *S. sanguis* is a gram-positive coccus with alpha-hemolytic colonies.

Sugar fermentation was done to further differentiate between the *Streptococcal* species. Each species ferments different sugar. For example, *S. mutans* ferments mannitol, lactose, raffinose, sorbitol, trehalose arginine, and to voges-prausker tests, but *S. pyogenes* do not respond to mannitol, raffinose, sorbitol and to voges-prausker tests. Similarly, the sugar fermentation test results were recorded in Table 1. Further, the five isolated species were based on antigenic types confirmed through latex agglutination test. *S. pyogenes* and *S. mutans* belonged to group A, *S. sanguis* to group H, *S. mitis* and *S. salivarius* to viridans (Table 1).

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Table 2. Prevalence of identified *Streptococcus species* in the saliva samples of each group

Isolated bacteria	Covid recovered group (n=25)	Prevalence percentage	Non-covid group (n=25)	Prevalence percentage	P value
S. sanguis	2	8%	1	4%	0.230
S. mitis	4	16%	2	8%	0.080
S. mutans	25	100%	23	92%	0.003
S. salivarius	3	12%	1	4%	0.349
S. pyogenes	21	84%	19	76%	0.155

Table 3. Confirmation of *Streptococcus mutans* and *Streptococcus pyogenes* using PCR

Isolated bacteria	Covid recovered group (n-25)			Non-covid group (n=25)			P value
	Biochemical and antigenic test	PCR	Confirmation percentage	Biochemical and antigenic test	PCR	Confirmation percentage	
S. mutans	25	20	80%	23	19	82.6%	0.638
S. pyogenes	21	18	85.71%	19	17	89%	0.483

Of the 25 saliva samples collected from the COVID-recovered patient group, 25 *S. mutans* strains, 21 *S. pyogenes* strains, 4 *S. mitis*, 3 *S. salivarius* and 2 *S. sanguis* strains were identified using microbiological, biochemical, and antigenic tests. The prevalence percentage was 100% for *S. mutans*, 84% for *S. pyogenes* strains, 16% for *S. mitis*, 12% for *S. salivarius*, and 8% for *S. sanguis* strains (Table 4, Graph 1). Similarly, of the 25 saliva samples collected from the non-covid patient group, 23 *S. mutans* strains, 19 *S. pyogenes* strains, 2 *S. mitis*, 1 *S. salivarius*, and 1 *S. sanguis* strains were identified/ The prevalence percentage was 92% for *S. mutans*, 76% for *S. pyogenes* strains, 8% *S. mitis*, 4% for *S. salivarius* and *S. sanguis* strains. (Table 2, Fig. 1).

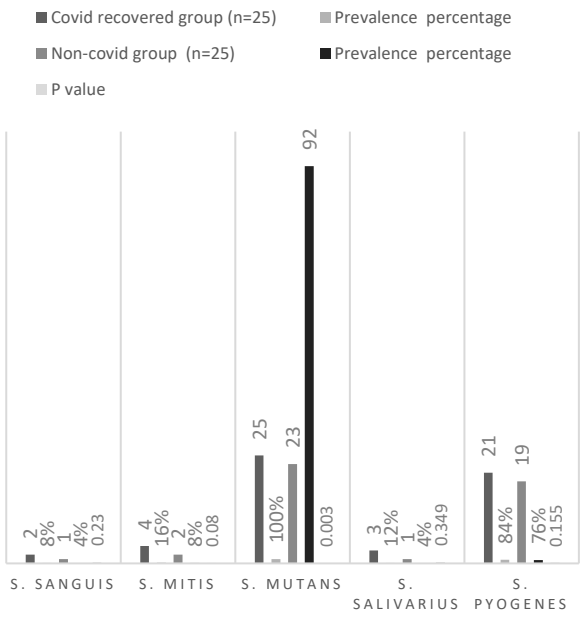

Figure 1. Prevalence of identified *Streptococcus species* in the saliva samples of each group

The species confirmation was done using PCR. Out of the 25 identified *S. mutans* strains from the samples, 20 could be confirmed using PCR. Hence, the confirmation percentage was 80%. Similarly, 18 strains out of the 21 *S. pyogenes* could be confirmed using PCR, leading to a conformation percentage of 85.71%. Following the same procedure, in the non-covid group, 19 *S. mutans* strains and 17 *S. pyogenes* strains were confirmed with PCR with a confirmation percentage of 82.6% and 89%, respectively, with a significant p-value of 0.638 and 0.438 (Table 3).

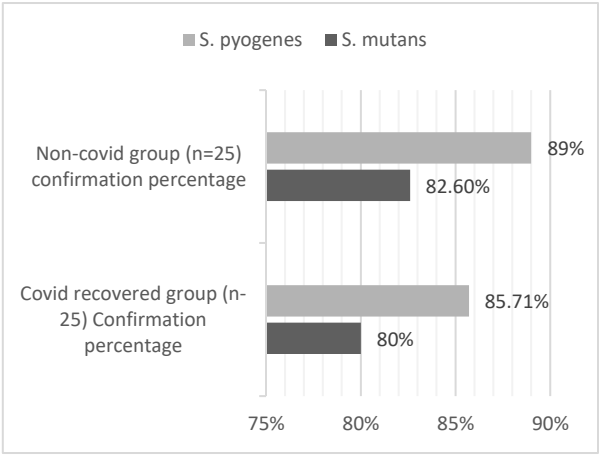

Figure 2. Confirmation of *Streptococcus mutans* and *Streptococcus pyogenes* using PCR

The isolated *Streptococcal strains* were subjected to an antibiotic sensitivity test using Kirby-Bauer's disc diffusion method. Eight antibiotics were used, which are the commonly prescribed antibiotics by dentists. The antibiotics used were Azithromycin, Clindamycin, Clarithromycin, Doxycycline, Erythromycin, Ciprofloxacin, Cefuroxime, and Amoxicillin Clavulanic acid. From the covid-recovered patient group, the *S. mutans* strains showed the maximum resistance to Azithromycin and Erythromycin, where 84% of strains were resistant, and the least resistant was towards Amoxicillin Clavulanic acid. Similarly, the *S. pyogenes* strains showed the maximum resistance to Erythromycin, where 95.23% of strains

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were resistant, and the least resistance was towards Cefuroxime with 76.19%. *S. sanguinis* and *S. mitis* recorded 100% resistance to Azithromycin and Ciprofloxacin. The resistance percentage of all the isolated strains is recorded in Table 4. Likewise, from the non-covid patient group, the *S. mutans* strains showed the maximum resistance to Doxycycline, where 65.21% of strains were resistant, and the least resistant was towards Cefuroxime. Similarly, the *S. pyogenes* strains showed the maximum resistance to Doxycycline and Erythromycin, where 63.15% of strains were resistant, and the least resistance was again towards Cefuroxime with 36.84%. *S. sanguis* and *S. mitis* recorded no resistance to most antibiotics. The resistance percentage of all the isolated strains from the non-covid group is recorded in Table 4.

Table 4. Resistance percentage of the isolated strains from the saliva samples of the covid recovered and the non-covid group toward the commonly prescribed antibiotics by dentists (in %; N=35)

Covid recovered group	Isolated bacteria	Number of isolated strains used	Resistance percentage to the commonly prescribed antibiotics by dentist (in %)							
			Az	Cd	Cth	Dx	Ery	Cpf	Cfx	Amx-Ca
	S. sanguis	2	100	50	50	50	100	100	50	50
	S. mitis	4	100	50	50	50	75	100	50	25
	S. mutans	25	84	76	68	76	84	72	80	60
	S. salivarius	3	66.6	66.6	66.6	66.6	66.6	66.6	66.6	33.3
	S. pyogenes	21	90.47	80.95	80.95	85.71	95.23	85.71	76.19	85.71
Non-Covid Group	*S. sanguis*	1	0	0	0	0	0	0	0	0
	S. mitis	2	50	50	0	100	0	0	0	0
	S. mutans	23	52.17	47.82	60.8	65.21	56.52	43.47	34.78	39.13
	S. salivarius	1	100	100	0	100	0	0	0	0
	S. pyogenes	19	57.89	47.36	52.63	63.15	63.15	57.89	36.84	42.10

Note: Az: Azithromycin; Cd: clindamycin; Cth: Clarithromycin; Dx: Doxycycline; Ery: Erythromycin; Cpf: Ciprofloxacin; Cfx: Cefuroxime; Amx-Ca: Amoxicillin Clavulanic acid

Discussion

Streptococcal infection is a common issue in almost all age groups of patients, irrespective of gender. Likewise, antibiotic resistance in *Streptococcus* has also risen in the last few decades. It is a well-known fact that the use of antibiotics leapt significantly during the COVID era. People took antibiotics irrespective of whether they were prescribed or not, which has given a significant boost to antibiotic resistance. This study primarily focused on antibiotic resistance in *Streptococcal* species affecting the oral cavity. It was discernible that the *Streptococci* strain isolated from the COVID recovered group showed more resistance to the antibiotics than those isolated from the non-covid group. In particular, *S. mutans* and *S. pyogenes* strains, the primary oral pathogenic bacteria, were resistant to most antibiotics tested in this study. Occurrences of co-infections were reported from hospitalized COVID-19 patients from UAE. Isolation and characterization of MDR strains of *Streptococcus* and *Candida* species were reported in the above study [21]. COVID-19 was linked with an increase in periodontal infections during the pandemic due to a compromised immune system, elevated levels of cytokines, DNA damage and increased virulence levels of periodontitis-causing bacteria[22]. Antibiotic overuse can select microorganisms with resistance. Antibiotics are often recommended for dental infections or prophylaxis before treatments. Failure to finish an antibiotic course may leave more resistant bacteria. These bacteria can multiply and cause drug resistance. Some oral bacteria resist antibiotic classes, making treatment more challenging [23, 24]. *S. mutans* and *Porphyromonas gingivalis*, which cause tooth cavities and periodontal disease, are multidrug-resistant. Conjugation, transformation, and transduction can provide oral bacteria resistance genes. This lets resistance characteristics spread quickly among oral bacteria species. Biofilms on tooth surfaces or oral tissues protect these bacteria from antibiotics. Biofilms allow bacteria to share genetic material and communicate, developing resistance [25].

Antibiotic resistance has been affected by the COVID-19 pandemic, but the exact type and amount of this effect may depend on several factors. Antibiotics have been used often, sometimes wrongly, to treat secondary bacterial infections in COVID-19 cases during the pandemic. There is no doubt that the COVID-19 pandemic has affected antibiotic resistance; however, the precise magnitude and type of this influence may vary based on several different conditions. The use of antibiotics to treat secondary bacterial infections in COVID-19 patients has been widespread during the pandemic, and there have been instances when they have

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been used unnecessarily [26, 27]. Moreover, antibiotics have been used as a preventative measure in certain instances, notably in patients in critical condition. Both the inappropriate use of antibiotics and their excessive usage has played a role in developing antibiotic resistance. Since the major emphasis was on COVID-19, attention and resources may have been diverted away from surveillance efforts for antibiotic resistance and antimicrobial stewardship programs. Because of this lack of monitoring and oversight, there is a possibility that incorrect antibiotic prescribing practices will rise, which will further contribute to developing bacteria resistant to antibiotics[28-30].

The pandemic's burden on healthcare systems may have contributed to challenges in providing adequate care for bacterial diseases. This strain included hospitals and clinics that were already operating at capacity. This could lead to a delay in the detection and treatment of bacterial infections, which has the potential to result in more severe cases and an increase in the usage of antibiotics. Because of the extensive usage of personal protective equipment (PPE) during the pandemic, such as masks and gloves, there is a possibility that antibiotic-resistant bacteria will be able to thrive[28-30]. The spread of microorganisms that are resistant to treatment can occur when PPE is misused, reused, or not disinfected adequately. There is a possibility that antibiotic research and development efforts have slowed considerably because of the redirection of money and attention into COVID-19 research. Therefore, the development of novel antibiotics and alternative treatments necessary to tackle illnesses resistant to antibiotics may be hampered [31, 32].

Many studies have suggested possible ways to address the above problem. They recommended a suitable antibiotic usage policy to address the above problem. Tan et al. 2023 reported the difference in oral flora and dysbiosis between COVID-19 and non-covid patients, particularly in elderly patients [33]. Also, Mihra et al., 2020 and Bessa et al., 2022 suggested using a combination of antibiotics for managing oral health and probiotics for combatting dysbiosis [34, 35]. Reports suggest using antimicrobial peptides (AMPs) for treating oral infections instead of chlorhexidine and calcium hydroxide, which dentists have traditionally used.[36] An Indian study reported using Baicalein (5,6,7-trihydroxyflavone) to control the antibiotic-resistant strains of *S. mutans,* which had high virulence and biofilm-causing capacity. Also, it was reported that it did not affect normal commensals of the oral cavity[36]. Reports suggest employing alternative methods like nanoparticles or nanotechnology to control oral infections or using herbal medicines or photodynamic therapy to reduce antibiotics[37,38]. New modified safety regulations can be implemented in dental hospitals and clinics to reduce infections. There can be increased awareness of the use of antibiotics both in dentists and patients to reduce the burden of MDR *Streptococcus* species[39].

Nanopore technology has great potential in combating MDR bacteria. This technology helps rapidly identify the bacteria and the resistant gene through rapid analysis and whole genome sequencing of the bacterial genetic material. Compared to traditional sequencing methods, nanopore sequencing can be more affordable, especially for large-scale projects. Nanopore technology can directly sequence RNA without the need for reverse transcription, preserving information about RNA modifications[40].

The data obtained can be used to design specific antimicrobial drugs to destroy MDR strains and are beneficial for rapid diagnostics and research. This technology, along with other *in silico* methods, can also be used to surveillance MDR strains and predict possible outbreaks and pandemics[41]. The portable nature of nanopore sequencers makes them suitable for use in remote settings, enabling on-site testing and monitoring of bacterial resistance patterns. The data generated can aid in the development of new antibiotics and therapies by revealing potential targets for drug design[42]. However, compared to other techniques like Illumina sequencing, nanopore sequencing has higher error rates, which might make data interpretation more difficult. Sophisticated bioinformatics techniques and significant computational resources may be needed due to the volume of data generated. Even if the technology is advancing, certain high-throughput sequencing techniques may still outperform it in terms of throughput. Long reads may be produced, although shorter reads and varying degrees of precision are also possible. The quality of the input sample might have a considerable impact on the outcomes; hence it is important to handle and prepare the sample carefully. Some laboratories may find it difficult to implement and maintain nanopore technology since it requires specific knowledge.[43] Overall, nanopore technology improves our ability to detect, monitor, and respond to MDR bacteria, potentially reducing their influence on public health; nevertheless, its limits must be carefully considered in the context of specific applications.

Conclusion

In conclusion, there has been a significant rise in the MDR strains of *Streptococcus* species in India and globally. In our study, COVID-19-recovered patients had more *Streptococcus* species isolated from their oral cavity than strains isolated from the healthy controls. Our study also concluded that the strains isolated from COVID-19 recovered patients were resistant to more antibiotics than those isolated from non-covid patients. Notably, more resistant *S. mutans* and *S. pyogenes* strains are alarming. Hence, alternative strategies have to be opted for dealing with such virulent MDR strains.

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References

1. Kumar A, Singh R, Kaur J, Pandey S, et al. Wuhan to World: The COVID-19 Pandemic. *Front Cell Infect Microbiol.* 2021 30; 11:596201.
2. Brandini DA, Takamiya AS, Thakkar P, Schaller S, et al. Covid-19 andoral diseases: Crosstalk, synergy or association? *Revs Med Virol*. 2021;31: e2226.
3. Yumoto H, Hirota K, Hirao K, et al. The Pathogenic Factors from Oral *Streptococci* for Systemic Diseases. *Int J Mol Sci.* 2019;20: 4571.
4. Imai K, Tanaka H. SARS-CoV-2 Infection and significance of oral health management in the era of "the new normal with COVID-19". *Int J Mol Sci.* 2021;22 :6527.
5. Di Pierro F, Iqtadar S, Mumtaz SU, Bertuccioli A, et al. Clinical effects of *Streptococcus salivarius* K12 in hospitalized COVID-19 patients: Results of a preliminary study. *Microorganisms.* 2022;10:1926.
6. Langford BJ, Soucy JR, Leung V, So M et al. Antibiotic resistance associated with the COVID-19 pandemic: a systematic review and meta-analysis. *Clin Microbiol Infect.* 2022; 9: S1198-743X (22)00610-3.
7. Kariyawasam RM, Julien DA, Jelinski DC, et al. Antimicrobial resistance (AMR) in COVID-19 patients: a systematic review and meta-analysis (November 2019-June 2021). *Antimicrob Resist Infect Control.* 2022;11:45.
8. Alves-Barroco C, Rivas-García L, Fernandes AR, Baptista PV. Tackling Multidrug Resistance in Streptococci - From Novel Biotherapeutic Strategies to Nanomedicines. *Front Microbiol.* 2020; 11:579916.
9. Farid H, Khan M, Jamal S, Ghafoor R. Oral manifestations of Covid-19-A literature review. *Revs Medical Virol.* 2022;32: e2248.
10. De Bruyn A, Verellen S, Bruckers L, et al. Secondary infection in COVID-19 critically ill patients: a retrospective single-centre evaluation. *BMC Infect Dis.* 2022;22:207
11. Pourajam S, Kalantari E, Talebzadeh H, et al. Secondary bacterial infection and clinical characteristics in patients with COVID-19 admitted to two intensive care units of an academic hospital in Iran during the first wave of the pandemic. *Front Cell Infect Microbiol*. 2022; 12:784130.
12. Abranches J, Zeng L, Kajfasz JK, Palmer SR, et al. Biology of Oral *Streptococci*. *Microbiol Spect.* 2018;6:6-5.
13. Rehman S. A parallel and silent emerging pandemic: Antimicrobial resistance (AMR) amid COVID-19 pandemic. *J Infect Public Health*. 2023;16:611-617.
14. Seethalakshmi PS, Charity OJ, Giakoumis T, Kiran GS, et al. Delineating the impact of COVID-19 on antimicrobial resistance: An Indian perspective. *Sci Total Environ*. 2022; 818:151702.
15. Langford BJ, Soucy JR, Leung V et al. Antibiotic resistance associated with the COVID-19 pandemic: a systematic review and meta-analysis. *Clin Microbiol Infect*. 2023;29:302-309.
16. Arshad AR, Ijaz F, Siddiqui MS, Khalid S, et al. COVID-19 pandemic and antimicrobial resistance in developing countries. *Discoveries (Craiova).* 2021;9: e127.
17. Hoshino T, Kawaguchi M, Shimizu N, Hoshino N, et al. PCR detection and identification of oral streptococci in saliva samples using gtf genes. *Diagn Microbiol Infect Dis*. 2004;48:195-199.
18. Mondal DP, Panda CS, Rath S, Mohanty N. Comparative study on the efficacy of advanced and conventional methods for detection of *Streptococcus mutans* in the saliva of dental caries patients. *Indian J Public Health Res Dev*. 2018; 11: 1098-1022
19. Spellerberg B, Brandt C. Laboratory diagnosis of *Streptococcus pyogenes* (group A streptococci) 2016 Feb 10. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: Basic biology to clinical manifestations [Internet]. Oklahoma City (OK): University of Oklahoma Health Sciences Center; 2016-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK343617/
20. Rath S, Dubey D, Sahu MC, Padhy RN. Surveillance of ESBL-producing multidrug-resistant *Escherichia coli* in a teaching hospital in India. *Asian Pac J Trop Dis*. 2014;4:140-149.
21. Senok A, Alfaresi M, Khansaheb H, et al. Coinfections in patients hospitalized with COVID-19: A descriptive study from the United Arab Emirates. *Infect Drug Resist.* 2021; 14:2289-2296.
22. Ghosh A, Joseph B, Anil S. Does periodontitis influence the risk of COVID-19? A scoping review. *Clin Exp Dent Res.* 2022;8:1011-1020.
23. Salgado Peralvo AO, Kewalramani N, Mateos-Moreno MV. Antibiotic Resistance: The Silent Pandemia (in the COVID-19 Era). *Int Dent J.* 2023;73:328-329.
24. Rodríguez-Fernández A, Vázquez-Cancela O, Piñeiro-Lamas M, Figueiras A, Zapata-Cachafeiro M. Impact of the COVID-19 pandemic on antibiotic prescribing by dentists in Galicia, Spain:

A Quasi-experimental approach. *Antibiotics (Basel)*. 2022;11:1018.
25. Rath S, Bal SCB, Dubey D. Oral biofilm: Development mechanism, multidrug resistance, and their effective management with novel techniques. *Rambam Maimonides Med J*. 2021;12: e0004.
26. Wong C. Covid-19 pandemic linked to antibiotic resistance in pneumonia bacterium. *New Sci*. 2023;257:14.
27. Khoshbakht R, Kabiri M, Neshani A, Khaksari MN, et al. Assessment of antibiotic resistance changes during the Covid-19 pandemic in northeast of Iran during 2020-2022: an epidemiological study. *Antimicrob Resist Infect Control*. 2022;11:121.
28. Subramanya SH, Czyż DM, Acharya KP, Humphreys H. The potential impact of the COVID-19 pandemic on antimicrobial resistance and antibiotic stewardship. *Virus Dis*. 2021;32:330-337.
29. Parisini A, Boni S, Vacca EB, Bobbio N et al. Impact of the COVID-19 pandemic on epidemiology of antibiotic resistance in an intensive care unit (ICU): The Experience of a North-West Italian Center. *Antibiotics (Basel)*. 2023;12:1278.
30. Subagdja MFM, Sugianli AK, Prodjosoewojo S, Hartantri Y, Parwati I. Antibiotic resistance in COVID-19 with bacterial infection: laboratory-based surveillance study at single tertiary hospital in Indonesia. *Infect Drug Resist*. 2022; 15:5849-5856.
31. Smith DRM, Shirreff G, Temime L, Opatowski L. Collateral impacts of pandemic COVID-19 drive the nosocomial spread of antibiotic resistance: A modelling study. *PLoS Med*. 2023;20: e1004240.
32. Zeshan B, Karobari MI, Afzal N, Siddiq A, Basha S, et al. The Usage of Antibiotics by COVID-19 Patients with Comorbidities: The Risk of Increased Antimicrobial Resistance. *Antibiotics (Basel)*. 2021;11:35.
33. Tan L, Zhong MM, Liu Q, Chen Y, et al. Potential interaction between the oral microbiota and COVID-19: a meta-analysis and bioinformatics prediction. *Front Cell Infect Microbiol*. 2023; 13:1193340.
34. Mishra S, Rath S, Mohanty N. Probiotics-A complete oral healthcare package. *J Integr Med*. 2020;18:462-469.
35. Bessa LJ, Botelho J, Machado V, Alves R, Mendes JJ. Managing Oral Health in the Context of Antimicrobial Resistance. *Int J Environ Res Public Health*. 2022;19:16448.
36. Griffith A, Mateen A, Markowitz K, et al. Alternative Antibiotics in Dentistry: Antimicrobial Peptides. *Pharmaceutics*. 2022;14:1679.
37. Vijayakumar A, Sarveswari HB, Vasudevan S, Shanmugam K, et al. Baicalein Inhibits *Streptococcus mutans* Biofilms and Dental Caries-Related Virulence Phenotypes. *Antibiotics (Basel)*. 2021;10:215.
38. Rath S, Dubey D, Babu SK, Pattanaik S. Employing antimicrobial nanoparticles to reduce oral biofilm medicine. *J Med Pharm Allied Sci*. 2021; 16 : 1671-1681
39. Satyarup D, Jena M, Nagarajappa R, Upasana Dhar U, Rath S. Perception of risk, fear, compliance, and social stigma associated with COVID-19 pandemic among dental patients of Bhubaneswar, Odisha. *Palestinian Med Pharm J*. 2023;8: 289-296.
40. Deng Q, Cao Y, Wan X, Wang B, Sun A, et al. Nanopore-based metagenomic sequencing for the rapid and precise detection of pathogens among immunocompromised cancer patients with suspected infections. *Front Cell Infect Microbiol*. 2022; 12:943859.
41. Rath S. In silico prediction of pandemics: A computational tool to complement real-world assessment. *Epidemiol Health System J*. 2024;11:x-x doi:10.34172/ehsj.26086
42. Zhang L, Huang W, Zhang S, Li Q, Wang Y, Chen T, Jiang H, Kong D, Lv Q, Zheng Y, Ren Y, Liu P, Jiang Y, Chen Y. Rapid detection of bacterial pathogens and antimicrobial resistance genes in clinical urine samples with urinary tract infection by metagenomic nanopore sequencing. *Front Microbiol*. 2022; 13:858777.
43. Delahaye C, Nicolas J. Sequencing DNA with nanopores: Troubles and biases. *PLoS One*. 2021;16: e0257521.