ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 12 | December - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

STUDY OF MICRO-ENVIRONMENTAL CHANGE OF NASOPHARYNX AND OROPHARYNX IN COVID INFECTED PATIENTS AND ITS EFFECT ON OTHER KNOWN COMMENSALS AND PATHOGENIC BACTERIA

Medical Microbiology

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ABSTRACT

Commensal microflora colonizing the oropharynx of normal individual acts as adeterrent factor for oropharyngeal and nasopharyngeal inflammation by preventing pathogenic bacteria causing localized infection / inflammation. Any change in the commensal population by various methods like iatrogenic intervention or overwhelming viral infections etc. are the likely reason for respiratory tract infection which if left untreated can lead to morbidity of different magnitudes. The present study intends to find out if any change of oral or pharyngeal microflora occurs in individuals with COVID infections. **Study area** Murshidabad Medical College and hospital, Berhampore, West Bengal. **Study population** Patients with positive reports for COVID 19 , diagnosed by RT PCR method , admitted in the critical care wards of Murshidabad Medical College and hospital. **Study design** Hospital based observational study.

KEYWORDS

INTRODUCTION

The human upper respiratory tract is the major portal of entry for infectious droplet or aerosol-transmitted microorganisms, including the 2019 emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Unique microbial communities reside on the mucosal surfaces of the respiratory tract, which conditions the host defence both directly and indirectly . In addition, acute respiratory infections by virus are associated with changes in microbial composition in the respiratory, which in turn may alter subsequent immune function against secondary bacterial infection or alter the dynamics of inter-microbial interactions, thereby enhancing the proliferation of potentially pathogenic bacterial species.

With rise in the number of coronavirus disease 2019 (COVID-19) cases worldwide and because of apparently absent cross-protective immunity from related viral infections, SARS-CoV-2 transmissibility became high, due to widespread person-to-person transmission [2]. Both asymptomatic and symptomatic patients may transmit the virus [3].

Studies have explored the relationship between SARS-CoV-2 infection and the bacterial community within the respiratory tract. Therefore, we investigated whether the presence of the virus in the respiratory tract might reflect alterations of the resident microbiota by studying the bacterial communities of SARS-CoV-2 infected patients . The respiratory tract is a constant balance of tolerance of commensal and non-invasive microbes and immune activation against pathogens. Possible mechanism by which viral infections might predispose infected hosts to secondary bacterial pneumonia is by altering the microbial composition of the upper respiratory tract, fostering enhanced growth of pathogens, and facilitating the subsequent entry of large bacterial loads into the lower respiratory tract .

METHODS

For this study, nasopharyngeal swab samples of the suspected COVID-19 patient's admitted in critical care unit were collected within the time span of January 2021 to November 2021. Trained medical professionals were used to collect the samples in the viral transport medium (VTM) transported to the lab following the ICMR guideline published by the Ministry of Health and Family Welfare, Government of India samples were tested by RT-PCR method as approved by Indian Council of Medical (ICMR), Government of India [4].

Patients with samples collected from known COVID-19 positive patients admitted in the critical care unit (i.e. who presented symptoms of acute respiratory infection who were admitted in Murshidabad Medical college & Hospital West Bengal India from January 2021 onwards) were included. Nasopharyngeal swabs were tested for RT-PCR based COVID-19 diagnosis [4].From the same patients, samples (throat swab) were collected separately (endotracheal suction for ventilated patient) and growth was obtained on blood agar and MacConkey agar. They were used to profile the nasopharyngeal microbial flora by vitek -2 automated method.

RESULTS

The microbial flora isolated from the samples collected from the covid 19 positive patients in all samples belonged to the following groups namely Klebsiella sp., Pseudomonas, Escherichia coli Actinobacteria, and Staphylococci. (18%) of the patients presented with mixed commensal growth. These findings were consistent with the high similarity shared by the noscomial bacterial pneumonia patients[10].

Table -1 Total Pathogens Isolated From Culture Positive Cases (n=78)

Type of infection	Bacteria Pathogens		Total
	Gram Positive Bacteria	Gram Negative Bacteria	
Early – onset	6	28	34
Late – onset	4	40	44
Total	10	68	78

Pathogens Isolated (n=78)

Table – 2 Pathogens Isolated In Early – Onset infection (n=34)

Pathogens			Number of Isolates	Percentage (%)
Gram Negative bacteria	Non – Fermenters	Pseudomonus aeruginosa	24	31
		Acinatobacter	6	8
	Entero bacteriaceae	Klebsiella pneumoniae	14	18
		Citrobacter	4	5
		E,coli	15	20
		Proteus	5	6
Gram – positive bacteria Total	Staphylococcus aureus		10	12
			78	100

Table – 3

Early – onset Pathogens			Number of Isolates	Percentage (%)
Gram Negative bacteria	Non Fermenters	Acinatobacter	1	3
		Pseudomonas	9	26.5
	Enterobacteriaceae	E.Coli	8	23
		Klebsiella	7	20

		Proteus	1	3
		Citrobacter	2	6
Gram Positive bacteria	Staphylococcus aureus		6	18.5

Table – 4 Pathogens Isolated In Late – Onset infection (n=44)

Late – onset Pathogens			Number of Isolates	Percentage (%)
Gram Negative bacteria	Non Fermenters	Acinatobacter	5	11.5
		Pseudomonas	15	34
	Enterobac teriaceae	E.Coli	7	16
		Klebsiella	7	16
		Proteus	4	9
		Citrobacter	2	4.5
Gram Positive bacteria	Staphylococcus aureus		4	9

Table -5 Etiological Agents of in Different ICUs

Pathogens	Number of Isolates			Total
	TSU	CCU	ITU	
Acinetobacter	1	1	4	6
Pseudomonas aeruginosa	2	9	13	24
Klebsiella pneumonia	2		12	14
Citobacter		2	3	4
Escherichia coli	4	1	10	15
Proteus			5	5
Staphylococcus aureus		2	8	10
Total	9	14	55	78

Table - 6 Antibiotic Resistance Pattern (%) in Early – onset infection

Gram negative bacteria		PT	AK	CIP	CTX	CTZ	MRP
Non fermenters	Acinetobacter (1)	100	100	100	100	100	0
	Pseudomonas (9)	66.6	77.7	44.5	77.7	55.5	11.1
Enteroba cteriaceae	Klebsiella (7)	28.5	0	28.5	57.14	71.4	0
	Citrobacter(2)	50	50	50	50	100	
	E.coli(8)	62.5	75	12.5	62.5	75	25
	Proteus(1)	100	0	0	100	0	0
Gram positive bacteria	M	LZ	CIP	AZ	AK	TP	
S.aureus(6)		16.6	16.6	83.3	83.3	83.3	

PT-PIPRACILLIN TAZOBACTUM
AK-AMIKACIN
CIP- CIPROFLOXACIN
CTX-CEFOTAXIME
CTZ- CEFTAZIME
MRP-MEROPENEM
LZ-LINEZOLID
TP-TEICOPLANIN
AZ-AZITHROMYCIN

Table - 7 Antibiotic Resistance Pattern (%) in Late –Onset infection

Gram negative bacteria		PT	AK	CIP	CTX	CAZ	MRP
Non fermenters	Acinetobacter (5)	80	80	100	100	100	20
	Pseudomonus (15)	53	46	53	90	33	26
Enterobacteri aceae	Klebsiella sp (7)	28	28	57	57	71	0
	E.coli(7)	85	28	57	71	85	28
	Citrobacter (2)	50	0	50	50	100	0
	Proteus sp(4)	50	50	25	50	50	25
Gram positive bacteria		M	LZ	CIP	AZ	TP	TP
S.aureus(4)		25	0	100	75	75	0

DISCUSSION

In this study, SARS-CoV-2 infection was associated with a different profile of the nasopharynx bacterial community analyzed at COVID-19 diagnosis, which is when the patients viral disease of the respiratory tract. This suggests that microbial compositional alterations do occur in response to SARS-CoV-2 or that SARS-CoV-2 led to superadded bacterial colonization which acted as a source for bacterial pneumonia in these patients.

As nasopharyngeal swabs can be a reasonable proxy for lung samples [5], Nasopharyangeal flora act as a nidus for lung infection by the following methods

(1) Microaspiration in semi conscious or unconscious patients
(2) Patients on PPI
(3) Recumbent patients
(4) The pathogenesis of infection has been attributed to direct mucosal/epithelial damage by virus, increased bacterial colonization of the upper and lower respiratory tracts (URT and LRT, respectively), and dysregulation of immune responses, which all lead to increased susceptibility to secondary bacterial infections. The mechanisms underlying post-viral bacterial infections are complex and include multifactorial processes mediated by interactions between viruses, bacteria, and the host immune system. Studies have demonstrated that unique microbial communities reside on the mucosal surfaces of the respiratory tract, which have both direct and indirect effects on host defense against viral infections[6][12]. Thus, we did find pathogenic bacterial growth which was significantly higher in SARS-CoV-2 infected patients [6][11][12].

The limitations of this study

(1) SARS-CoV-2 infections were defined by RT-PCR positivity, (the possibility is that false negative RT-PCR results have biased the grouping of patients in our study)
(2) Patients with symptoms but negative RT PCR reports were not included. Although RT-PCR performed on a nasopharyngeal swab remains the reference diagnostic test [4], patients with negative RT-PCR results could be highly probable COVID-19 cases based on positive chest X ray or CT findings [7]. But again CT findings can be absent, especially in patients with early and/or mild disease [9]. Therefore, we can assume some covid positive cases might have been missed in our study. Further investigation of SARS-CoV-2 infected patients with detailed viral shedding data and sequential respiratory samples is warranted.
(3) Only hospitalized cases on invasive mechanical ventilation were included in the study hence this may not represent the population as a whole [8] .

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