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A Comparative Study of Vaccinated and Non-Vaccinated Covid-19 Cases Against Klebsiella Pneumoniae

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ABSTRACT

A Comparative study of COVID- 19 vaccinated individuals against bacterial co-infections could explore valuable insights such as the effectiveness of vaccines in preventing secondary infections, comparative analysis of bacterial infection rates between vaccinated and unvaccinated populations and the COVID-19 vaccines impact the incidence of specific bacterial infections, such as *Klebsiella pneumoniae*. The start of the COVID-19 pandemic brought about new healthcare challenges, with secondary bacterial infections in hospitalized patients posing additional health threats. In the present cohort study, we analyzed the infection rates of *Klebsiella pneumoniae* between vaccinated and unvaccinated groups of COVID-19 vaccination for long COVID patients from July 2022 to June 2024. A total of 254 patients in and around Madurai district were collected based on patient demographics such as age, sex, underlying health conditions, vaccination status and district wise distribution of the samples were processed as per standard microbiological techniques

Keywords: *UTI Urinary Tract Infections- K.pn - Klebsiella pneumoniae*

1. INTRODUCTION

Klebsiella pneumoniae are reported among neonates/children,adults and elderly as it causes wide range of nosocomial as well as community associated infections such as urinary tract infections and Bacteremia and Septicemia. The present study focused that the effect of COVID-19 vaccinated and non-vaccinated cases against the bacteria *Klebsiella pneumoniae* from different types of samples among three districts. A total of 254 *Klebsiella pneumoniae* isolates were isolated from the diagnostic centres, hospitals located in Madurai, Sivagangai and Dindigul.

2. METHODOLOGY

Patient details were collected and recorded and the collection of clinical specimens such as, Urine, Pus, Stool, Throat swab, Pulmonary secretions such as sputum, were collected from the patients with suspected *Klebsiella pneumoniae* infection and blood samples are collected if sepsis or bacteremia is suspected. The samples were collected from 3 districts such as Madurai, Sivagangai and Dindigul and the specimens are to be collected from the post COVID-19 recovered patients as well. Isolation and Identification and confirmation of *Klebsiella pneumoniae* from clinical samples by using selective media like MacConkey agar and biochemical test like oxidase, catalase and indole test and performed antibiotic susceptibility testing to determine the antibiotic resistance profile

the Metaverse can guarantee the integrity, security, and transparency of data management and transactions, according to a

Collection of Data

Specimen collection and Patient consent form

The patients and their attendees are instructed to read the informed consent form and clearly explained and the signature is taken in the informed consent form. The clinical data's and past history of COVID illness, COVID vaccination details and another Co-morbid illness are collected and recorded in a case report form(CRF). The specimens are collected from three districts - Madurai, Sivagangai and Dindugul.

The collection of specimens in Madurai are from few selected reputed hospitals and clinical laboratories. They are Arthur Ashirvatham hospital, Saravana Multi Speciality Hospital (Oomachikulam),Sundar laboratory (K.Pudur), Hi-Tech laboratory (Tallakulam), Sundaram laboratory(IyerBunglow),VinithaLaboratory(B.B.Kulam),AnnamalaiLaboratory(Therkuvasal), Neogen Laboratory(Bypass road) and Madurai BioCare lab (Sellur). The specimen collection in Sivagangai was done from Omsakthi Hospital and for Dindugul district collection was done from Muthu Multispecialty Hospital, Nilakottai and Pearl Laboratory, Nilakottai. **Sivagangai**

Analysis of samples

The study was conducted over a period of two years from June 2022 to July 2024. The toal number of 254 *Klebsiella pneumoniae* isolates were collected and analyzed from clinical specimens The samples were collected as per the collection process listed above, the samples were transferred and stored in the laboratory at 37^0 C in an incubator and based on the types of samples, it is transferred to an enrichment media before incubation. The basal media and specific media are prepared, sterilized and autoclaved. The petri plates are also sterilized in a hot air oven and after preparing the media are poured in a sterile petri plate and after solidification, the specimens are streaked in an appropriate plate and after streaking, the plates are kept in an incubator at 37^0 C for 24 – 48 hours. Next day, the streaked plates are observed and colonies subjected to the staining process such as Gram stain and capsule stain. The gram stain results were recorded in the research log note and CRF form. Then, the bio-chemical analyses were done. On 3rd day, the biochemical analysis and culture results were read and recorded and confirmed.

3. MORPHOLOGICAL AND BIOCHEMICAL IDENTIFICATION OF *K. PNEUMONIAE*

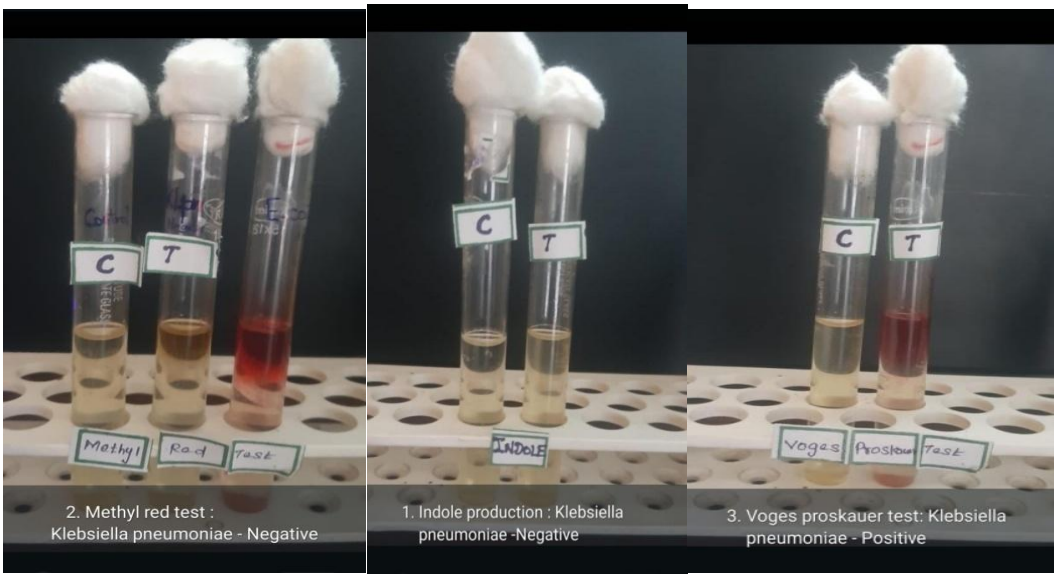
The isolates were subjected to routine morphological and biochemical tests and the results are presented in Table-1. The isolate was confirmed as gram negative, non-motile, catalase positive, oxidase negative with acid production in TSI, mannitol positive, Indole negative, Methyl red negative, Voges Proskauer positive and citrate positive. Presumptive identification of the isolate as *K. pneumoniae* was confirmed with these tests. Following these tests the isolate was confirmed by its plating in the selective medium namely MacConkey agar and its characteristic pink color colonies were confirmed. Age wise distribution was finalized from the total cases (254) the result was showed that the age group ratio from 26 – 45 was highest (66/254) (25.98%) and least in the age group of new born and infants (1- 12months) (4/254) (1.57%) which is depicted in table 2. This study reports the infection in both sexes. Table 3 reports the sex wise distribution among the total cases(254),females are prone to be highly infected one (159/254)(62.6 %) when compared to male with lesser percentage (95/254),(37.40%).

This study reveals the district wise distribution in Table 4. Among the 3 districts namely Madurai, Sivagangai, Dindugul, high percentage (162/254) (63.8%) of infection was reported from Madurai specially from South Madurai. Different types of specimens were collected from the three districts concluded that a greater number of urine specimens (159/254) (62.5%) were collected and next to urine, upper respiratory samples such as pus samples were collected with the percentage of (59/254) (23.22%), sputum (8/254) (3.14%) and blood with (24/254) (9.44%), stool samples with (3/254) (1.18%), semen with (1/254) (0.3%). These results are depicted in table 5. Table 6 reports the occurrence of the isolate in vaccinated and non-vaccinated individuals. Based on culture growth, *Klebsiella pneumoniae* was reported in 150 vaccinated cases and 104 non-vaccinated.

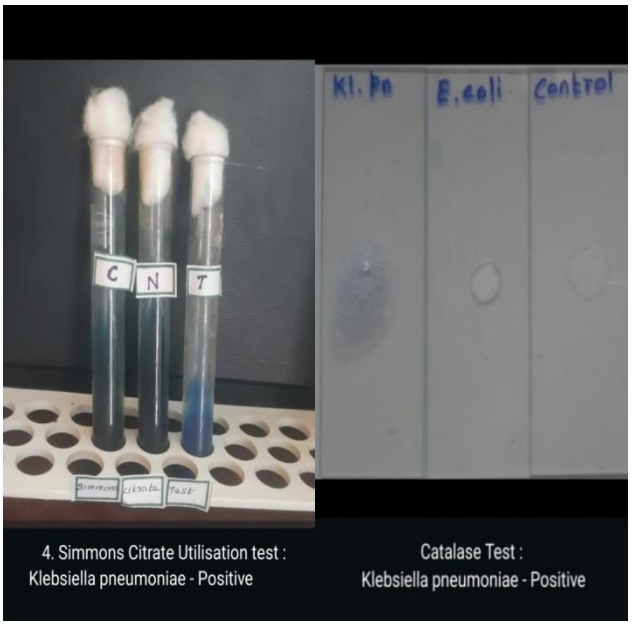
Table -1- Morphological and Biochemical Identification of *K. pneumoniae*

S.No	**Tests**	**Results**	**Inference**
1	Gram staining	Bacilli in pairs	Gram negative
2	Motility	No movement	Non-motile
3	Catalase	Bubbles formation	Positive
4	Oxidase	Absence of dark purple color	Negative

		formation on the discs	
5	Triple sugar iron test	Acid /Acid- Yellow color formation	Positive
6	Mannitol	Yellow color formation	Positive
7.	Indole production	No cherry red color formation	Negative
8.	Methyl red test	No color formation	Negative
9.	Voges Proskauer test	Pink color formation	Positive
10.	Citrate Utilization test	Color change of the medium from green to blue	Positive



IMViC Test



Colony morphology of Klebsiella pneumoniae

Table- 2. Age-wise Distribution

S.no	Particulars	Vaccinated	Non vaccinated
1.	0-28 days– New born	-	7
2.	1–12 Months – Infant	-	4
3.	1-3 Years- Toddlers	-	16
4.	4-5 Years– Pre school	-	8
5.	6-12 Years –Children	-	17
6.	13-17Years –Adolescent	-	7
7.	18-25 Years–Young adult	23	10
6.	26-45 Years – Adult	66	14
7.	46-59 Years–Middle Adult	28	04
8.	Above 60 Years – Old	33	17
	Total	**150**	**104**

Table-3- Sex-wise Distribution

S.No	Particulars	Vaccinated N=150	Non vaccinated N=104	Total
1.	Male	55	40	95 (37.40%)
2.	Female	95	64	159 (62.6%)
	Total	**150**	**104**	

S.no	Particulars	Vaccinated N=150	Non vaccinated N=104
1.	Madurai	92	71
2.	Sivagangai	33	26
3.	Dindugul	25	07

	Total	**150**	**104**

Table-4- District-wise Distribution

Table-5- Specimen –wise Distribution

S.no	Particulars	Vaccinated	Non vaccinated
1	Urine	85	74
2	Blood	10	14
3	Pus	47	12
4	Stool	01	02
5	Sputum	06	02
6	Semen	01	00
7	Throat swab	00	00
8	Vaginal swab	00	00
	Total	**150**	**104**

S.No	Name of the organism	Vaccinated	Non vaccinated
1.	*Klebsiella pneumoniae*	**150**	**104**

Table-6- Culture Growth

4. DISCUSSION

The present study reveals that COVID-19 vaccinated individuals have a higher frequency of secondary bacterial co-infections, particularly *Klebsiella pneumoniae*,compared to non-vaccinated individuals. This observation challenges the conventional understanding of vaccine efficacy in preventing secondary infections. COVID-19 vaccines may modulate the immune system,potentially increasing susceptibility to secondary bacterial infections. Our study suggests that COVID-19 vaccination may not provide protection against secondary bacterial co-infecions, particularly *Klebsiella pneumoniae* and also the complex interplay between viral and bacterial pathogens might be influenced by vaccination, leading to an increased risk of secondary infections. Most of the infected cases were isolated from Madurai district especially in the South zone of Madurai has more number of infected cases. The major reason for the high infection rate in the south zone of Madurai is because of overcrowding and crowded spaces and closed spaces between the houses and with poor ventilation was the increased risk of infection. Hence, preventive measures like social distancing must be emphasized in the community. Symptomatic and severe cases spread the infection more than asymptomatics and mild cases, mass screening of the community at the primary level for the symptoms like fever, cough, sore throat, running nose, and breathlessness help in early diagnosis and isolation.resulting in higher secondary infection.and more number of populations as well as houses are most near those affected with co-morbid conditions. Any other co-morbid condition for post COVID patients have the high frequency of Bacterial infection. (Hanada S etal.,2018). In additional , More isolates were from the Urine specimens and higher infections were found in 21 -30 age group with 31.49% of cases. Old age adults and children with immune compromised also a major reason for more number of bacterial infection (Cherry J, Harrison G, Kaplan *et.al*., 2019)

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