Microbes and Infectious Diseases 2024; 5(2): 441-450

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Originalarticle

Co-infection with viruses among critically-ill SARS-CoV2 patients in a tertiary hospital in Egypt: Incidence and effect on patients outcome

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ARTICLE INFO

Article history:
Received 1 March 2024
Received in revised form 18 March 2024
Accepted 24 March 2024

Keywords:
Co-infections
Super-infection
Biofire filmarray pneumonia panel
SARS-CoV-2

ABSTRACT

Background: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection could be associated with other co/super-infections that worsen the outcome. In our hospital, the burden of viral co-infections with SARS-CoV-2 is unknown. We aimed to identify the lower respiratory tract viral pathogens causing co-infections among SARS-CoV-2 patients, and to elucidate their outcome. **Methods:** We enrolled 147 patients with SARS-CoV-2 infection. Lower respiratory viral co-infection was identified in non-repetitive respiratory specimens by BiofireFilmArray Pneumonia Panel. Culture and Vitek-2 were used to identify bacterial and/or fungal super-infections in co-infected patients whose outcome was evaluated. **Results:** Of 147 enrolled patients, 29 had viral co-infection. The most common co-infection was by Influenza A (34.2%), Rhinovirus/Enterovirus (31.6%), other Coronaviruses (13.2%). Seventeen (58.6%) patients developed super-infection. Streptococcus pneumoniae was the most frequently isolated super-infection pathogen (17.2%). Mortality occurred in 41.40% of patients with co-infection which was higher compared to the group without co-infection (32.2%) but with no statistical significance, p: 0.463. In the co-infection cohort, the deceased patients' hemoglobin (Hb), platelets count, C-reactive protein (CRP), and procalcitonin (PCT) levels were significantly different compared to survived ; 9.14 ± 1.23, 185.15 ± 91.42, 169.31 ± 94.91, and 2.42± 2.91 vs 10.65± 2.15 mg/dl, 249.0± 100.69 x103/µL, 49.88 ± 59.57 mg/l, and 0.44 ± 0.55 ng/ml, p value 0.044, 0.006, <0.001, and 0.012, respectively. Additionally, the deceased patient had a considerably longer ICU stay (25.75 ± 23.6 vs. 11.80 ± 11.19 days, p: 0.066). **Conclusion:** In SARS-CoV-2 patients, co-infection with other respiratory viruses is linked to increased mortality. The Hb and platelet counts of the deceased patients are higher than those of the survivors, while their PCT and CRP levels are lower.

Introduction

Viruses, bacteria, fungi, and protozoa are examples of numerous pathogens that frequently infect the same host concurrently or consecutively. This phenomenon is defined as co/super-infection [1]. Typically, co/super-infection increases the severity of symptoms, complicates the course of the disease and worsens the outcome [2]. Clinical diagnosis of co-infection in patients hospitalized with corona virus disease 2019 (COVID-19), caused

DOI:10.21608/MID.2024.273956.1831
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by the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) is challenging, and diagnosis requires laboratory testing. In addition, super-infection by bacterial and fungal pathogens among COVID-19 patients exceeded 15% [1,2].

Early detection of other viral pathogens optimizes patient management, especially in critically ill SARS-CoV-2 patients, avoids prolonged antibiotic courses, limits evolution of multidrug-resistant pathogens, improves the patients' outcome, and saves hospital resources [3-6].

Although culture methods have been considered the gold standard for identification of infectious pathogens, molecular diagnostics have been recently included as a reliable diagnostic tool for respiratory pathogens, due to their short turn-around-time and good sensitivity in detecting fastidious organisms and viruses [4]. Syndromic FilmArray Pneumonia panel (BioFire diagnostics LLC, Salt Lake City, UT, USA) can detect 15 common bacteria, 3 atypical bacteria, and 8 viruses that cause community and hospital-acquired lower respiratory tract infection, as well as 7 antibiotic resistance genes, in approximately 1 hour [5-7]. To our knowledge, few publications covered the challenge of co/super-infection among SARS-CoV-2 patients in Egypt [8]. In our hospital, the burden of viral co-infections with SARS-CoV-2 is unknown.

This study aimed to identify the lower respiratory viral pathogens causing co-infections among SARS-CoV-2 patients, and to describe their outcome.

Material and methods

Study design and setting

Between March 2020 and February 2021, 147 mini-bronchoalveolar lavage (BAL) and endotracheal aspirate (ETA) samples from SARS-CoV-2 patients admitted in ICU in a certain tertiary hospital in Egypt were enrolled in this cross-sectional analytical analysis. Ethical approval was given by the Research Ethics Committee of the Faculty of Medicine, Cairo University (N61-18).

Diagnosis of SARS-CoV-2 infection

For detection of SARS-CoV-2, nasopharyngeal swabs were collected, immersed in viral transport medium (the universal transport medium (UTM) from Copan Diagnostics) and immediately refrigerated till testing. Viral RNA extraction was done by following the manufacturer's instructions and utilizing the QIAMP VIRAL RNA micro kit (Qiagen, Hilden, Germany) with internal PCR control. The VIASURE SARS-CoV-2 Real-Time PCR detection kit (CerTest, BIOTEC, Spain) was utilized to detect COVID-19 in a one-step real-time RT format. Using particular primers and a fluorescently labeled probe, reverse transcription and the subsequent amplification of target sequence ORF1 ab and N genes happened simultaneously.

Diagnosis of viral co-infection

Testing by BiofireFilmArray Pneumonia Panel (FAPP) was done according to the guidelines provided by the manufacturer (BioFire diagnostics LLC, Salt Lake City, UT, USA) for detection of viral pathogens [9,10].

Diagnosis of bacterial/fungal super-infection

For detection of bacterial and fungal pathogens, a direct Gram stain was performed and examined immediately. Bronco-alveolar lavage (BAL), sputa, and endotracheal tube aspiration (ETA) specimens were evaluated by Gram stain regarding the quality of specimen before culturing and testing by FAPP. Specimen with over 10 epithelial cells per low power field on conventional Gram stain were excluded and rejected. The accepted good quality samples were applied to 5% sheep blood agar, MacConkey agar, chocolate agar, and Sabouraud Dextrose agar (Oxoid, UK). The samples were then incubated for 24 hours at 37°C and a week at room temperature on the Sabouraud Dextrose agar (Oxoid, UK). Antimicrobial susceptibility testing and pathogen identification were conducted using the Vitek-2 automated system (BioMérieux, Marcy l'Etoile, France).

Evaluation of patients' clinical outcome

Clinical outcome was assessed using 28 days mortality, length of ICU stay.

Statistical data analysis

Version 25 of IBM SPSS for Windows (Armonk, New York: IBM Corp) was used to conduct the statistical analysis, with a significant difference at p-value less than 0.05. Categorical variables were described in the form of frequencies and percentages, whereas non parametric

continuous variables were described as the mean ± SD. Data normality was tested using Shapiro-Wilk Test of Normality, then Mann-Whitney test was done to analyze the differences and relationships between two unrelated continuous variables. For categorical variables, the Chi-square ($\chi 2$) test was used, when more than 20% of cells have predicted frequencies less than 5, Fisher's exact test was utilized.

Results

In this cross-sectional analytical study, we enrolled 147 patients admitted to our ICU with SARS-CoV-2 infection, of them 29 (19.7%) proved to have co-infection with other respiratory viruses. In the latter, the age ranged from 49 to 88 years with an average age of 68.86 ±11.73 years old, 19 patients (65.5%) were males. Only one viral pathogen was identified in 21 specimens; Influenza A was the most predominant of them, followed by Rhinovirus/Enterovirus (34.2% and 31.6%, respectively). Two viral pathogens in 7 specimens and 3 viral pathogens in one specimen were identified, **(Table 1)** and **(Figure 1)**.

During the ICU course, it was found that 17 out of 29 (58.6%) patients developed bacterial and/ or fungal super-infection. As illustrated in **(Table 2)**, *Streptococcus pneumoniae* was the most frequently isolated pathogen, followed by *Escherichia coli* ,*Haemophilus influenzae*, *Candida albicans*, *Klebsiella pneumoniae*, *and Acinetobacter baumannii*. Despite the higher length of ICU stay prior to specimen collection in the super-infection patients compared to patients without super-infection, 20.46 ± 12.18 vs 16.68± 8.0 days, yet this finding was statistically insignificant, p value: 0.565. As well, no other significant association was found between any of the studied parameters and the incidence of super-infection **(Table 3)**.

Regarding patients` outcome, mortality occurred in 12 (41.40%) patients with co-infection which was higher compared to the other group, (118 (32.2%), but without a statistically significant difference (p=0.463). In the deceased patients, with respiratory viruses` co-infection, hemoglobin concentration, platelets count, C-reactive protein (CRP), and procalcitonin (PCT) were significantly different compared to survived patients; 9.14 ± 1.23, 185.15± 91.42, 169.31 ± 94.91, and 2.91± 2.42 vs 10.65± 2.15 mg/dl, 249.0± 100.69 $x10^3/\mu L$, 59.57 ± 49.88 mg/l, and 0.55 ± 0.44 ng/ml, p value 0.044, 0.006, <0.001, and 0.012 respectively. Additionally, the length of ICU stay was also significantly higher in the deceased patient, 25.75± 23.6 vs 11.80±11.19 days, p value: 0.066. Number of days with mechanical ventilation didn't differ between deceased patients and survived patients, p-value: 0.952 **(Table 4)**.

Table 1. List of the type and number of viral pathogens. Abbreviations: FAPP: BiofireFilmArray pneumonia panel, RSV: Respiratory syncytial virus.

Number of specimens	Viruses detected by FAPP	Number of viral pathogens
1.	Corona virus	1
2.	Human Rhinovirus/Enterovirus	1
3.	Human Rhinovirus/Enterovirus	1
4.	Coronavirus, Human Rhinovirus/Enterovirus	2
5.	Coronavirus, Human Rhinovirus/Enterovirus	2
6.	Influenza A	1
7.	Influenza A	1
8.	Adenovirus, Influenza A, Influenza B	3
9.	Influenza A	1
10.	Influenza A	1
11.	Influenza A	1
12.	Human Rhinovirus/Enterovirus	1
13.	Influenza A	1
14.	Human Rhinovirus/Enterovirus	1
15.	Human Rhinovirus/Enterovirus	1
16.	RSV	1
17.	Influenza A	1
18.	Human Rhinovirus/Enterovirus	1
19.	Influenza A	1
20.	Human Rhinovirus/Enterovirus, Influenza A	2
21.	Influenza A	1
22.	RSV	1
23.	RSV, Human Rhinovirus/Enterovirus	2
24.	Human Rhinovirus/Enterovirus/ Influenza A	2
25.	Corona virus, Influenza A	2
26.	Human Rhinovirus/Enterovirus	1
27.	Adenovirus, RSV	2
28.	Corona virus	1
29.	Adenovirus	1

Table 2. Super-infection with bacterial and fungal pathogens among the co-infected cohort

Type of pathogen		Number of patients (%)
Gram negative bacteria *13 (44.8%)*	*Hemophilus influenza*	3 (10.3%)
	Escherichia coli	3 (10.3%)
	Acinetobacter baumannii	2 (6.9%)
	Klebsiella pneumonia	2 (6.9%)
	Enterobacter cloacae	1 (3.4%)
	Serratia marcescens	1 (3.4%)
	Pseudomonas aeruginosa	1 (3.4%)
Gram positive bacteria 5 (17.2%)	*Streptococcus pneumoniae*	5 (17.2%)
Fungi *4 (13.7%)*	*Candida albicans*	3 (10.3%)
	Candida tropicalis	1 (3.4%)

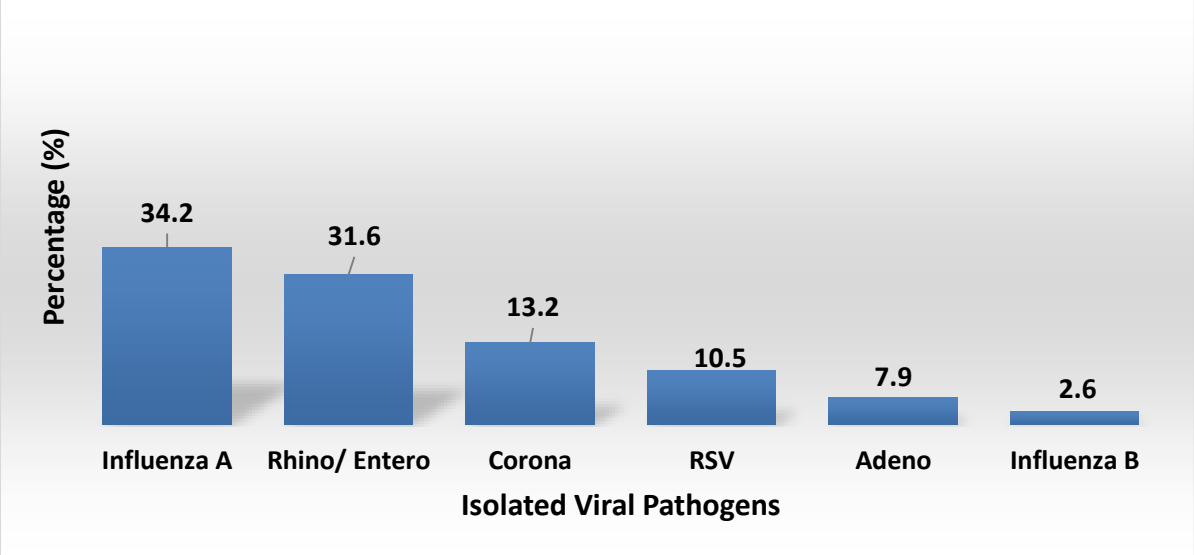
Table 3. Association between super-infection incidence and other studied variables. Abbreviations: LOS: Length of ICU stay; APACHE: Acute physiology and chronic health evaluation; Hb: Hemoglobin; WBCs: White blood cells; PLT: Platelets; CRP: C-reactive protein; PCT: Procalcitonin

		Mean	SD	Minimum	Maximum	p-value
Age (years)	No Super-infection	73.55	10.42	56	92	0.089
	Super-infection	65.82	11.82	44	88	
LOS prior to specimen collection (days)	No Super-infection	16.68	8.00	-16	45	0.565
	Super-infection	20.46	12.18	-5	64	
APACHE II Score at admission	No Super-infection	25.83	11.65	7	40	0.092
	Super-infection	19.29	8.56	8	35	
Hb (g/Dl)	No Super-infection	10.27	1.62	7.9	12.4	0.427
	Super-infection	9.68	2.11	6.3	14.5	
WBC (10^3 µ/l)	No Super-infection	11.28	4.64	5.7	23.0	0.785
	Super-infection	10.79	4.77	6.4	26.0	
PLTs (10^3 /µL)	No Super-infection	209.50	115.10	28	413	0.144
	Super-infection	270.41	101.71	142	520	
D-DIMER (mg/l)	No Super-infection	2.28	1.85	0.41	6.00	0.896
	Super-infection	2.19	1.73	0.15	6.00	
CRP (mg/l)	No Super-infection	108.42	79.55	7	228	0.820
	Super-infection	99.88	109.97	8	298	
PCT (ng/ml)	No Super-infection	1.52	2.90	0.02	10.00	0.703
	Super-infection	1.19	1.61	0.04	6.00	
LOS (days)	No Super-infection	23.42	16.88	2	64	0.253
	Super-infection	32.12	21.52	10	92	

Table 4. Association between the results of laboratory tests and mortality in the studied patients. Abbreviations: Hb: Hemoglobin; WBCs: White blood cells;PLT: Platelets; CRP: C-reactive protein; PCT: Procalcitonin; APACHE: Acute physiology and chronic health evaluation; LOS: Length of ICU stay; MV: Mechanical ventilation.

Laboratory investigations		Mean	SD	Minimum	Maximum	p-value
Hb (g/Dl)	Survived	10.56	2.15	6.3	14.5	0.044
	Deceased	9.14	1.23	6.8	11.0	
WBC (10^3µ/l)	Survived	10.39	4.92	5.7	26.0	0.447
	Deceased	11.74	4.34	6.4	23.0	
PLTs count (10^3 /µL)	Survived	294.00	100.69	140	520	0.006
	Deceased	185.15	91.42	28	316	
D-DIMER (mg/l)	Survived	2.07	2.01	0.15	6.00	0.598
	Deceased	2.42	1.41	0.41	6.00	
CRP (mg/l)	Survived	49.88	59.57	7	228	<0.001
	Deceased	169.31	94.91	12	298	
PCT (ng/ml)	Survived	0.44	0.55	0.02	1.60	0.012
	Deceased	2.42	2.91	0.06	10.00	
APACHE II	Survived	17.56	9.28	7	40	0.007
	Deceased	27.46	9.01	8	40	
LOS (days)	Survived	11.80	11.19	1	45	0.066
	Deceased	25.75	23.61	3	64	
Days on MV	Survived	27.50	20.00	3	64	0.952
	Deceased	28.00	19.22	2	66	

Figure 1. Frequency distribution of viral co-infections.



Discussion

Co-infection with SARS-CoV-2 and other viruses has been associated with disease severity, morbidity and mortality [11]. Adding the BioFire FilmArray Pneumonia Panel to standard laboratory methods could identify co-infection with other viruses and negate bacterial infections, which could have a positive impact on antimicrobial stewardship in adult ICU patients [12]. In our ICU patients, viral co-infection was identified in 29 out of 147 (19.7%) of SARS-CoV-2 patients. This is in keeping with

previous studies highlighting the prevalence of co-infection between SARS-CoV-2 and other respiratory viruses [13-15]. Of particular concern is the high prevalence of co-circulation of influenza (Flu) and SARS-CoV-2, as it may cause a more severe coronavirus 2019 disease and increase the predicted fatality [1]. In our study, Influenza A virus was the most detected, co-infecting SARS-CoV-2 patients, followed by Rhinovirus/Enterovirus. This is in agreement with previous reports from Egypt and worldwide [14-17]. In Egypt, among patients with viral co-infection, 82.7% were co-infected with Flu A and 17.3% with Flu-B [16]. These co-infections were associated with high rates of hospitalization, morbidity and mortality [18, 19]. This is consistent with our findings of high death rates in patients with co-infections, which may be attributed to a compromised immune system in these patients. Kim EH et al. reported that compared with a single infection with SARS-CoV-2 or Influenza A virus, co-infections led to severe pneumonia and lung damage, in addition to lengthening the duration of the initial virus infection. Furthermore, co-infections in his animal model resulted in considerable peripheral blood lymphopenia, which decreased the responses of CD4+ T cells, neutralizing antibody titers, and total IgG against each virus.. They also proved that co-infections increased inflammatory cytokine and immune cell infiltration levels in bronchoalveolar lavage fluid [20].

The high frequencies of super-infection with bacterial and fungal pathogens, in SARS-CoV-2 patients, contributed to the unfavorable outcome. This confirms the high frequency of bacteria causing pneumonia among critically-ill SARS-CoV-2 patients, including Gram-ve bacteria with high prevalence of multidrug-resistant phenotypes [17, 21]. The predominance of Gram-negative bacilli is which is consistent with the global and Egyptian aetiology of hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) [22, 23]. Although our results were not statistically significant, days prior to sample collection and ICU stay were longer in patients with super-infection compared to other patients. Our study may have been underpowered to detect significance of these variables. It is noteworthy that previous studies by Bardi, et al., and Yoon SM, et al [24, 25] identified an association between bacterial super-infections and increased mortality rates and longer stays in ICUs.

In our deceased co-infected patients, we found significant variation in their admission laboratory results; hemoglobin, PLT count, PCT and CRP; compared to the survived patients. Elevated PCT and CRP were numerously mentioned as mortality predictors in SARS-CO-V2 infection [26-28]. In fact, greater inflammatory markers are often associated with illness severity and the emergence of lung lesions in the early stages of SARS-COV-2 infection [29]. Discordant to our result, Liu et al. [30] did not report a value of red blood cells and platelet counts in mortality prediction in their cohort. It was noticeable that the co-infected patients with other respiratory viruses did not meet his inclusion criteria. Unfortunately, several limitations applied to our study. First, it was done at a single tertiary hospital; consequently, it's possible that our findings can't be generalized to other hospitals with different settings. Second, as this study was retrospective in nature, some insufficient or absent data might have had an impact on the results. As, we couldn’t compare patients with co-infections with patients who had only the SARS-CoV-2 infection. Furthermore, information regarding the pattern of antimicrobial sensitivity testing and individuals with multidrug-resistant organisms was unavailable and unretrievable. In addition, there was insufficient data to compare the occurrence of super-infection in patients who were not co-infected with those who had viral co-infection. Third, this study included a relatively small sample size. We recommend doing other large multicenter prospective studies to validate our findings. Also, we recommend vaccination to prevent SARS-COV-2, seasonal flu, in addition to a recently approved RSV vaccine [31, 32] to avoid disease severity and complications.

Conclusion

The study identified a high rate of co-infection with viruses in critically-ill SARS-COV-2 patients. The most common co-infection virus was with influenza. Most of our patients had a complicated course and high mortality rate.

Conflict of interest disclosure

No conflict of interests to be declared.

Funding

This research is self-funded.

References

1- Hughes S, Troise O, Donaldson H, Mughal N, Moore LSP. Bacterial and fungal coinfection

among hospitalized patients with COVID-19: a retrospective cohort study in a UK secondary-care setting. Clin Microbiol Infect 2020; 26(10):1395-1399.

2- Zhu X, Ge Y, Wu T, Zhao K, Chen Y, Wu B, et al. Co-infection with respiratory pathogens among COVID-2019 cases. Virus Res 2020; 285:198005.

3- Trotter AJ, Aydin A, Strinden MJ, O'grady J. Recent and emerging technologies for the rapid diagnosis of infection and antimicrobial resistance. CurrOpin Microbiol 2019; 51:39–45.

4- Liu HH, Yaron D, Piraino AS, Kapelusznik L. Bacterial and fungal growth in sputum cultures from 165 COVID-19 pneumonia patients requiring intubation: evidence for antimicrobial resistance development and analysis of risk factors. Ann Clin Microbiol Antimicrob 2021 Sep 25; 20(1):69.

5- Bhat N, O'Brien K, Karron R, Driscoll A, Murdoch D. Use and evaluation of molecular diagnostics for pneumonia etiology studies 2012;54(Suppl 2):S153-8.

6- Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, Patel R. Syndromic panel-based testing in clinical microbiology. Clin Microbiol Rev 2017; 31(1):e00024-17.

7- Yoo IY, Huh K, Shim HJ, Yun SA, Chung YN, Kang OK, et al. Evaluation of the BioFireFilmArray Pneumonia Panel for rapid detection of respiratory bacterial pathogens and antibiotic resistance genes in sputum and endotracheal aspirate specimens. Int J Infect Dis 2020; 95:326-331.

8- Fahim M, AbdElGawad B, Hassan H, Naguib A, Ahmed E, Afifi S, et al. Epidemiology and outcome of influenza-associated infections among hospitalized patients with acute respiratory infections, Egypt national surveillance system, 2016-2019. 2021;15(5):589-598. doi:10.1111/irv.12867

9- Gad MA, El-Kholy A, Taher OM. Diagnostic role of FilmArray Pneumonia Panel compared to routine microbiological methods to identify pneumonia pathogens. Microbes and Infectious Diseases 2023; 4(2): 477-486.

10- Leber AL, Clinical Microbiology Procedures Handbook. 4th ed. ASM Press; 2016.

11- Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, et al. Bacterial and fungal co-infection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing. Clin Infect Dis 2020;71(9):2459-2468.

12- Buchan BW, Windham S, Balada-Llasat JM, Leber A, Harrington A, Relich R, et al. Practical comparison of the BioFireFilmArray Pneumonia Panel to routine diagnostic methods and potential impact on antimicrobial stewardship in adult hospitalized patients with lower respiratory tract infections. J Clin Microbiol 2020; 58:e00135-20.

13- Maaike C, Swets M, Russel C, Harrison E, Lone N, Girvan M, et al. SARS-CoV-2 co-infection with influenza viruses, respiratory syncytial virus, or adenoviruses. The lancet 2022; 399: 16.

14- Lai CC, Wang CY, Hsueh PR. Co-infections among patients with COVID-19: The need for combination therapy with non-anti-SARS-CoV-2 agents? J Microbiol Immunol Infect 2020 Aug; 53(4):505-512.

15- Chong YM, Chan YF, Jamaluddin MFH, Hasan MS, Pang YK, Ponnampalavanar S, et al. Detection of respiratory viruses in adults with suspected COVID-19 in Kuala Lumpur, Malaysia. J Clin Virol 2021 Dec; 145:105000.

16- Fahim M, Roshdy WH, Deghedy O, Kamel R, Naguib A, Showky S, et al. Epidemiology,

Disease Severity and Outcome of Severe Acute Respiratory Syndrome Coronavirus 2 and Influenza Viruses Coinfection Seen at Egypt Integrated Acute Respiratory Infections Surveillance, 2020-2022. Can J Infect Dis Med Microbiol 2022 Nov 17; 2022:7497500.

17- Scott SJ, Pfotenhauer B, Weiner JJ, Hilleshiem J, Khubbar M, Bhattacharyya S. Respiratory Pathogen Coinfections in SARS-CoV-2-Positive Patients in Southeastern Wisconsin: A Retrospective Analysis. Microbiol Spectr 2021 Oct 31; 9(2):e0083121.

18- Fahim M, ElSood HA, AbdElGawad B, Deghedy O, Naguib A, Roshdy WH et al. Adapting an integrated acute respiratory infections sentinel surveillance to the COVID-19 pandemic requirements, Egypt, 2020-2022. Public Health Pract (Oxf) 2023;5:100358.

19- Fahim M, Abd ElGawad B, Hassan H, Naguib A, Ahmed E, Afifi S, et al. Epidemiology and outcome of influenza-associated infections among hospitalized patients with acute respiratory infections, Egypt national surveillance system, 2016–2019. Influenza Other Respi Viruses 2021; 15:589–98.

20- Kim EH, Nguyen TQ, Casel MAB, Rollon R, Kim S, Kim Y, et al. Coinfection with SARS-CoV-2 and Influenza A Virus Increases Disease Severity and Impairs Neutralizing Antibody and CD4+ T Cell Responses. J Virol 2022;96(6):e0187321.

21- Meawed TE, Ahmed SM, Mowafy SMS, Samir GM, Anis RH. Bacterial and fungal ventilator associated pneumonia in critically ill COVID-19 patients during the second wave. J Infect Public Health 2021 Oct; 14(10):1375-1380.

22- Farag AM, Tawfick MM, Abozeed MY, Shaban EA, Abo-Shadi MA. Microbiological profile of ventilator-associated pneumonia among intensive care unit patients in tertiary Egyptian hospitals. J Infect Dev Ctries 2020; 14(2):153-161.

23- See I, Lessa FC, ElAta OA, Hafez S, Samy K, El-Kholy A, et al. Incidence and pathogen distribution of healthcare-associated infections in pilot hospitals in Egypt. Infect Control Hosp Epidemiol 2013; 34(12):1281-1288.

24- Bardi T, Pintado V, Gomez-Rojo M, Escudero-Sanchez R, Azzam Lopez A, Diez-Remesal Y, et al. Nosocomial infections associated to COVID-19 in the intensive care unit: clinical characteristics and outcome. Eur J Clin Microbiol Infect Dis 2021; 40:495–502.

25- Yoon SM, Lee J, Lee SM, Lee HY. Incidence and clinical outcomes of bacterial superinfections in critically ill patients with COVID-19. Front Med (Lausanne) 2023;10:1079721.

26- Zare ME, Wang Y, Nasir Kansestani A, Almasi A, Zhang J. Procalcitonin Has Good Accuracy for Prognosis of Critical Condition and Mortality in COVID-19: A Diagnostic Test Accuracy Systematic Review and Meta-analysis. Iran J Allergy Asthma Immunol 2020 Dec 19; 19(6):557-569.

27- Kumar A, Karn E, Trivedi K, Kumar P, Chauhan G, Kumari A, et al. Procalcitonin as a predictive marker in COVID-19: A systematic review and meta-analysis. PLoS One 2022;17(9):e0272840.

28- Assal HH, Abdel-hamid HM, Magdy S, Salah M, Ali A, Elkaffas RH, et al. Predictors of severity and mortality in COVID-19 patients. Egypt J Bronchol 2022;16: 18.

29- Malik P, Patel U, Mehta D, Patel N, Kelkar R, Akrmah M et al Biomarkers and outcomes of COVID-19 hospitalisations: systematic review and meta-analysis. BMJ Evid Based Med 2021;26(3):107-108.

30- Liu Y, DuaX, Chena J, Jina Y, PengaL, Wang HHX, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. J Infect 2020;81(1):e6-e12.
31- Egypt Today. Egyptian Health Ministry advise citizens to obtain seasonal flu vaccine. https://www.egypttoday.com/Article/1/118415/Egyptian-Health-Ministry-advise-citizens-to-obtain-seasonal-flu-vaccine.
32- Fairbank R. RSV wave hammers hospitals - but vaccines and treatments are coming. Nature2022.

Saleh MH, Gad MA, El-Kholy AA, Saleh L, MostafaZM, Abd-El-Aziz SH. Co-Infection with respiratory viruses among critically-ill SARS-CoV2 patients in a tertiary hospital in Egypt: Incidence and effect on patients outcome. Microbes Infect Dis 2024; 5(2): 441-450.