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Prevalence of bacterial co infections among Covid 19 patients in wasit province

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***Abstract*---**Background: The pandemic of severe acute respiratory syndrome coronavirus 2 raised the attention towards bacterial co-infection and its role in coronavirus disease 2019 (COVID-19) disease. This study aims to review and identify the prevalence of bacterial co-infection in Iraqi patients as bacterial co-infection played an important role in escalating the morbidity and mortality rate during previous viral outbreaks and pandemics of COVID-19. Materials and Methods: It was collected three hundred eighty clinical samples from covid-19 patients, in Iraqi patients from a period between November 2021 to January 2021, in the bacteriology Unit. Patients samples were included upper respiratory tract, 190 (50%) from Nasopharyngeal (NP) and 190 (50%) from and Oropharyngeal (OP) specimen included 320 (84.2%) male and 60 (15.8%) female, in addition patients samples age was ranged between 21-70 years old. Each specimen was cultured on Blood agar, MacConkey agar, Mannitol Salt Agar and Chocolate Agar plates to be isolated, then samples was identified using biochemical test. Result: According to morphological and biochemical tests among collected samples 80 (21.1%) were positive samples, while 300 (78.9%) were negative samples, positive samples included 61(76.2%) gram negative (Gr -ve) and 19 (23.8%) were gram positive (Gr +ve), positive samples were distributed as following, 50 (62.5%) samples *K. pneumonia*, 3 (3.8%) *Pseudomonas aeruginosa*, 5 (6.2%) *Acinetobacter baumanii*, 4 (5%) *Micrococcus,* 12 (15%) *Staphylococcus aureus,* 3 (3.8%) *Enterococcus cloacae* and 3 (3.8%) were *Aerococcus viridans*. negative samples distributed into 200 (66.6%) normal flora and 100 (33.4%) were contaminated samples. According to gender patients

samples included 320 (84.2%) male and 60 (15.8%) female, in addition patients samples age was ranged between 21-70 years old and mean of age was 40.9±2.0 standard deviation (SD). In positive samples *Klebsiella pneumonia* represented 50 (62.5%) of 80(100%) collected samples, while other bacteria were 3 (3.75%), 5 (6.25%), 4 (5%), 12 (15%), 3 (3.75%) and 3 (3.75%) for *Pseudomonas aeruginosa, Acinetobacter baumanii, Micrococcus, Staphylococcus aureus, Enterococcus cloacae* and *Aerococcus viridans* respectively. *K. pneumoniae* according to age of patients age ranged between 21-70, it was showed that 8(16%%) of patients were in age group (21-30), 9(18%%) of patients were in age group (31-40), 12(24%%) of patients were in age group (41-50), 13(26%%) of patients were in age group (51-60) and 8(16%%) of patients were in age group (61-70). In distribution of *K. pneumoniae* according to gender of patients the vast majority of patients were male as male represented 39 (78%) of the patients while females were represented 11(22%). Conclusion: current data revealed that there was high rate of bacterial coinfection in Wasit province.

***Keywords*---**Covid19, bacterial coinfection, Iraq

Introduction

Respiratory viral and bacterial infections contribute substantially to the global burden of morbidity and mortality. Such simultaneous infections with the flu virus or bacteria that cause pneumonia, tend to make the patient's condition critical (1). Although, critically ill patients rapidly develop acute respiratory distress syndrome and sepsis, leading to death from multiple organ failure. The main symptoms of COVID-19 are fever, fatigue, and dry cough. However, most patients have a good prognosis (2). Bacterial co-infections associated with other coronaviruses, such as SARS-CoV-1 and MERS-CoV, have been reported in association with pandemic viruses at rates of 20–30%, respectively (3). Bacterial co-infection is directly linked to increased morbidity and mor- tality from viral respiratory infections. Hospital admissions increase the risk of healthcare-associated infections (HCAI) which makes the disease more aggressive and difficult to treat, as well as inducing life-threatening complications and increasing the consumption of antibiotics, super infections and co-infections are commonly found in many respiratory dis-eases; viral infectious diseases and bacterial co-infections may be the cause of the increased mortality rate in patients infected with any viral infection (4).

Coinfection associated with viral pneumonia is the main cause of mortality and can considerably inhibit the host's immune system, which decreases the pharmacological response and makes the prognosis of the disease harmful (5). SARS-CoV-2 is a newly emerged pathogen that causes pneumonia with the possibility of worsening to hypoxic-type respiratory failure, organ failure, and acute kidney injury followed by myocarditis and thromboembolism. SARS-Cov-2 (COVID-19) leaves the body vulnera-ble to bacterial infections; however, this co-infection mechanism is not well understood but represents a threat to the

respiratory epithelium favoring bacteremia (6). A study carried out with ICU (Intensive Care Unit) patients in 88 countries showed that those patients who received at least one antibiotic during acute hospitalization, of these, more than half developed a secondary bacterial infection, requiring antibiotic therapy, in China, 95% of patients and in the United Kingdom 80% of patients received antibiotics (7). Antimicrobial resistance is seen as a major threat to public health, as well as to the economy and health security at the local and international levels. It is estimated that due to its spread across countries and continents the bacterial resistance increase will cause 10 million deaths annually by the year 2050, relevant advances have been achieved and determined by the national AMR programme which is guided by the WHO Global Laboratory AMR Surveillance System (GLASS) in Uganda. Using the WHONET software (8), ARM data management was installed at the surveillance sites with trained personnel to guarantee the quality of the data. Six major pathogens that cause resistance-related deaths (Escherichia coli, followed by Staphylococcus aureus, Kleb- siella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa) were responsible for 929,000 deaths from ADR and 3.57 million (2.62–4.78) ADR-related deaths in 2019 (9). Secondary infections predominantly involve a specific group of bacterial pathogens such as S. aureus, Staphylococcus pneumoniae, Streptococcus pyogenes, and Haemophilus influenza (10).

2-Materials and methods:

2-1-Samples Collection

It was collected 380 samples from COVID-19 patients, bacterial samples were isolated and identified using different culture media in addition to various biochemical test. Patient variables were assessed to be correlated with patients age and sex, the percentage of positive isolates among collected samples also was documented (11).

2-2 Isolation and identification of bacterial isolates

Each specimen was cultured on Blood agar, MacConkey agar, Mannitol Salt Agar and Chocolate Agar plates. The resultant colonies in these media were subcultured to be more tests for identification to the species level as described by Bergey's Manual for determinative Bacteriology. The isolates were identified on the basis of typical morphology by gram staining, coagulase test, Triple Sugar Iron Agar test (TSI) and the analytical profile index (API) system (12).

2-3 Data analyses

Patients variables were associated statistically using Statistical Package for the Social Sciences (SPSS) software and analyzed with analysis of variance (ANOVA) with the GEN STAT software package, and outcomes (screening and positivity for secondary co-infection, death) using the Mann–Whitney test for continuous variables and the Fisher exact test for categorical variables. A *P*-value of < 0.05 was considered statistically significant.

3-Results

3-1 Patients variables

A total of three hundred eighty clinical samples were collected from covid19 patients, according to Wasit University ethics committee, in Iraq from October 2021 to February 2021, in the bacteriology Unit. According to gender patients samples included 320 (84.2%) male and 60 (15.8%) female, in addition patients samples age was ranged between 21-70 years old and mean of age was 40.9±2.0 standard deviation (SD), table (1).

Table (1) Demographic of collected samples.

Collected samples Samples	type	No (%)
Source of samples	Nasopharyngeal (NP) swab	190 (50%)
	Oropharyngeal (OP) swab	190 (50%)
Gender	Male	320 (84.2%)
	Female	60 (15.8%)
Age mean±SD (40.9±2.0)	21-30	89 (23.4%)
	31-40	121 (31.9%)
	41-50	93 (24.4%)
	51-60	47 (12.4%)
	61-70	30 (7.9%)

3-2 Identification of *K. pneumonia*

To identifying bacterial isolates, a variety of physiological, morphological, and biochemical tests were used for *K. pneumonia* including colony morphology, cultural and biochemical characteristics on the basis of typical morphology on agar plates, catalase test, oxidase test, Triple Sugar Iron Agar test (TSI) and the analytical profile index (API) system.

3-2-1 Colony morphology

Bacterial isolates were identified after 24-48 hours of aerobic incubation on blood agar, Mannitol Salt Agar, Chocolate Agar and MacConkey medium agar plates at 37°C. *Klebsiella pneumonia* colonies on MacConkey agar were Lactose fermenting colonies that are pink in color, have a regular edge, and are spherical and mucoid in texture, and big size (figure 1), concerning enrichment The bacterial isolates were mucoid, big, white to grey, and nonhemolytic colony on a blood-agar media, which was used to distinguish Klebsiella from other bacteria that grow on MacConkey agar but heamolyses blood.

Figure (1): Mucoid colonies of *K. pneumoniae* grown on MaConkey agar for 24 hrs at 37°C.

3-2-2 Catalase and oxidase test

It have been grown *K. pneumonia* on blood agar, Mannitol Salt Agar, Chocolate Agar and MacConkey medium agar plates at 37°C. *Klebsiella pneumonia* colonies from MacConkey agar were tested for both catalase and oxidase test production, results were positive for catalase as there was obvious bubbles formation and negative for oxidase through there was no change of filter paper to blue color, figure (2), (3).

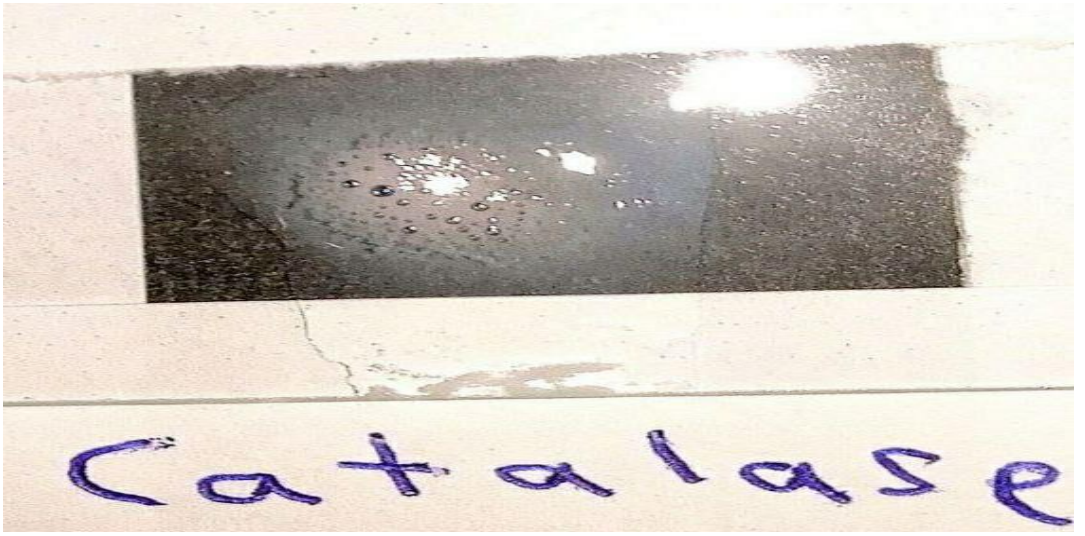

Figure (2) catalase test of *K. pneumonia*

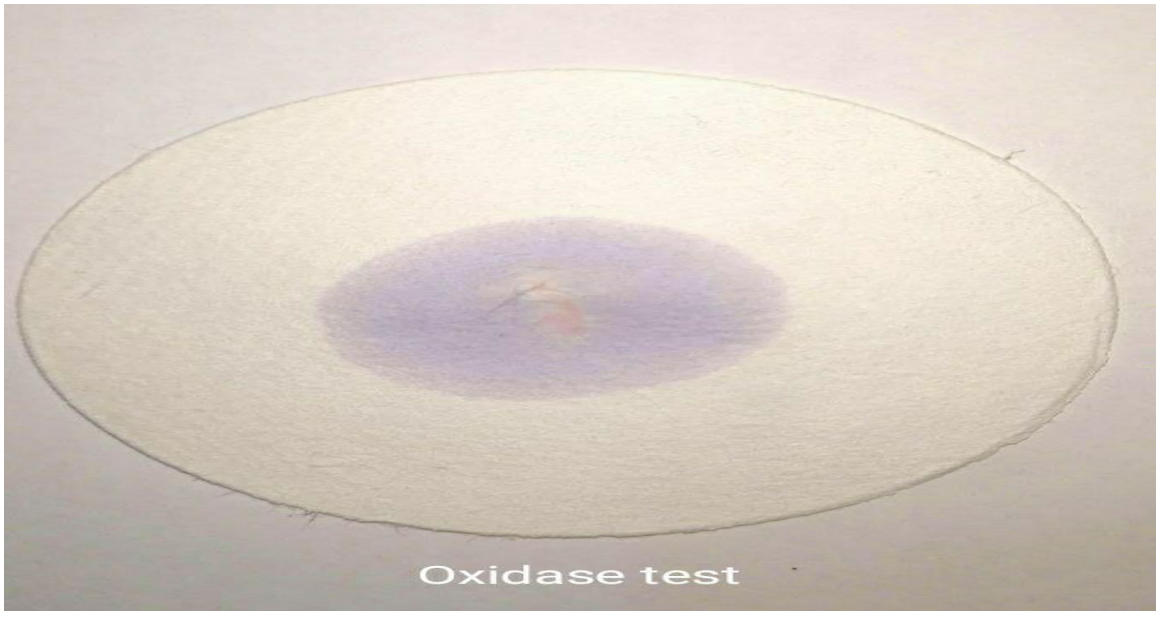

Figure (3) Oxidase test of *K. pneumonia*

3-2-3 Triple Sugar Iron Agar test (TSI)

It have been tested all 50 *K. pneumonia* isolate for Triple Sugar Iron Agar test (TSI), results were that Alkaline slant/alkaline butt (K/K) as red/red represent glucose, lactose, and sucrose non-fermenter, alkaline (K) slant, alkaline (K) buttom, negative for gas production and negative for H_2S production figure (4).

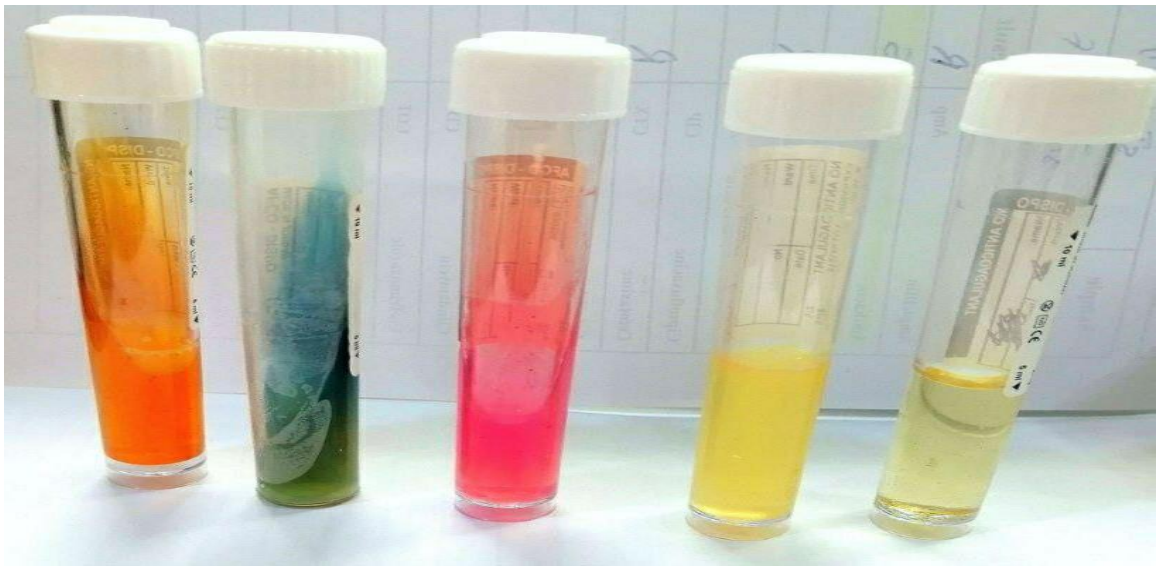

Figure (4): The TSI results of K. *pneumonia*.

3-2-4 API 20E system identification of *Klebsiella* isolates

As indicated in figure (5) , the results of biochemical tests for *Klebsiella* (50 isolates) were confirmed using the API 20E system . The results of all bacterial isolates were positive.

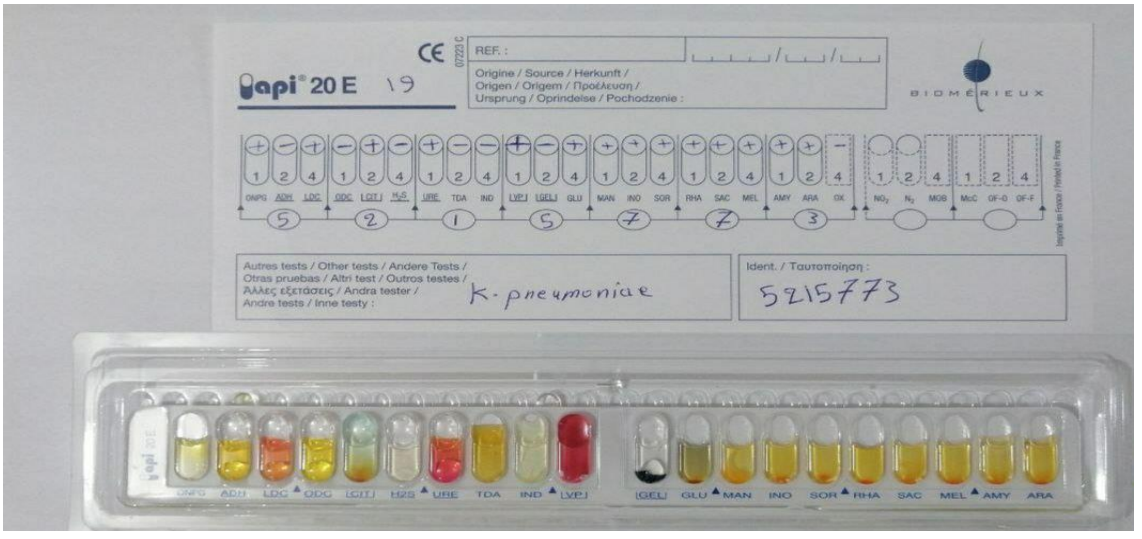

Figure (5): The analytic profile index 20E system for identifying K. *pneumonia*.

3-3 Percentage of positive isolates among collected samples

According to morphological and biochemical tests among collected samples 80 (21.1%) were positive samples, while 300 (78.9%) were negative samples, positive samples included 61(76.2%) gram negative (Gr -ve) and 19 (23.8%) were gram positive (Gr +ve), positive samples were distributed as following, 50 (62.5%) samples *K. pneumonia*, 3 (3.8%) *Pseudomonas aeruginosa*, 5 (6.2%) *Acinetobacter baumanii*, 4 (5%) *Micrococcus,* 12 (15%) *Staphylococcus aureus,* 3 (3.8%) *Enterococcus cloacae* and 3 (3.8%) were *Aerococcus viridans.* negative samples distributed into 200 (66.6%) normal flora and 100 (33.4%) were contaminated samples, table (2).

Table 2 Distribution of bacterial.

Sample collection Bacteri	**Gram -ve No (%)**	**Gram +ve No (%)**
Positive samples	*K. pneumonia* 50 (62.5%)	*P. aeruginosa*, 3 (3.8%)
	S. aureus 12 (15%)	*A. viridans* 3 (3.8%)
	A. baumanii 5 (6.2%)	*E. cloacae* 3 (3.8)
	Micrococcus 4 (5%)	
Negative samples	Normal flora	200 (66.6%)
	Contaminated samples	100 (33.4%)

3-4 Percentage of *Klebsiella pneumonia* among positive samples

In positive samples *Klebsiella pneumonia* represented 50 (62.5%) of 80(100%) collected samples, while other bacteria were 3 (3.75%), 5 (6.25%), 4 (5%), 12 (15%), 3 (3.75%) and 3 (3.75%) for *Pseudomonas aeruginosa, Acinetobacter baumanii, Micrococcus, Staphylococcus aureus, Enterococcus cloacae* and *Aerococcus viridans* respectively, table (3).

Table 3 Distribution of positive bacterial species.

Bacterial isolate	**No (%)**
Klebsiella pneumoniae	50 (62.5%)
Pseudomonas aeruginosa	3 (3.75%)
Acinetobacter baumanii	5 (6.25%)
Micrococcus	4 (5%)
Staphylococcus aureus	12 (15%)
Enterococcus cloacae	3 (3.75%)
Aerococcus viridans	3 (3.75%)
Total	80 (100 %)

3-5 Distribution of *K. pneumoniae* according to age of patients

In this regard patients age ranged between 21-70, it was showed that 8(16%%) of patients were in age group (21-30), 9(18%%) of patients were in age group (31-40), 12(24%%) of patients were in age group (41-50), 13(26%%) of patients were in age group (51-60) and 8(16%%) of patients were in age group (61-70), table (4) below.
Table (4): Distribution of *K. pneumoniae* in age group of patients

Age group (year)	Total No. of Isolates	Percentage
21-30	8	16%
31-40	9	18%
41-50	12	24%
51-60	13	26%
61-70	8	16%
Total	50	100%

3-6 Distribution of *K. pneumoniae* according to gender of patients

In this regard the vast majority of patients were male as male represented 39 (78%) of the patients while females were represented 11(22%), table (5).

Table (5): Distribution of *K. pneumoniae* in gender of patients

Gender of patients	Total No. of Isolates	Percentage
Male	39	78%
Female	11	22%
Total	50	100%

4-Discussion

A total of two hundred eighty clinical samples were collected from covid19 patients included upper respiratory tract, 140 (50%) from Nasopharyngeal (NP) and 140 (50%) from and Oropharyngeal (OP) specimen, among collected samples 80 (28.5%) were bacterial growth or culture. Current data were disagreed with Ahmed Hasan et al., (2021) (13) study aimed to determine the prevalence of bacteria and investigate the antibiotic resistance profile among clinical specimens of covid-19 at Azadi Teaching Hospital in Kirkuk, Iraq, in total, from clinical specimens were collected, (7.6%) and (11.6%) respectively of isolates samples were bacterial growth in culture, while our study agreed with other studies by Kadum, (2020) (14), Namratha et al. (2015) (15) and Nirwati et al. (2019) (16) reported such percentages as 4.03%, 17.36%, and 32.48%, these result is in line with the findings of the studies conducted by Al-Rubaye et al., (2016) (17) in Iraq. These differences in the mean prevalence rates among various studies could be related to differences in geographical location and hygienic practices of the population. *Klebsiella pneumonia* colonies on MacConkey agar were Lactose fermenting colonies that are pink in color, have a regular edge, and are spherical and mucoid in texture, and big size (18).

Klebsiella pneumonia colonies from MacConkey agar were tested for both catalase and oxidase test production, results were positive for catalase as there was obvious bubbles formation (19). It have been tested all 50 *K. pneumonia* isolate for Triple Sugar Iron Agar test (TSI), results were that Alkaline slant/alkaline butt (K/K) as red/red represent glucose, lactose, and sucrose non-fermenter, alkaline (K) slant, alkaline (K) buttom, negative for gas production and negative for H_2S production (20). Results of Kligler Iron Agar (KIA) test according to H2S

production and carbohydrates fermentation pattern that differentiates *Enterobacteriaceae* family from each other, this combination permits differentiation of Gram-negative bacilli by their ability to ferment Dextrose or Lactose, which produces color changes of the pH indicator in response to acid production during fermentation of the sugars. Dextrose concentration is 10% of the Lactose concentration. Ferric Ammonium Citrate and Sodium Thiosulfate mare indicators of hydrogen sulfide production. Phenol Red is the pH indicator. Sodium Chloride maintains the osmotic balance of the medium. Lactose nonfermenters as pH indicator (phenol red) changed the medium color from orange-red to yellow in the presence of acids. *K. pneumoniae* isolates able to produc acidic yellow slant and acid yellow bottom with H_2S and gas production, indicating glucose and lactose fermentation (21). In citrate test positive outcomes is an important phys-iological sign in order to diagnosing *Enterobacteriaceae* family, *K. pneumoniae* had a positive reactions in this test. *K. pneumoniae* that produced CO2, that reacting with medium components resulting in producing alkaline compounds, bromthymol blue must be turned from green to blue color as indicator of positive test (22). Indole negative in *Klebsiella* due to lacking of tryptophanase, so Kovac's reagent when added to broth which is usually free of indole, the red ring willnot formed (23). In urease test all isolates of *K. pneumonia* were positive as it was able to produce urease enzyme converting the yellow color to pink (20). It was showed that all *K. pneumoniae* isolates were positive for catalase test indicating presence of catalase enzyme able to convert hydrogen peroxide into H2O and O2 (24). In addition all *K. pneumoniae* isolates had been given a negative results regarding oxidase test, this may due to that *K. pneumonia* isolates lacking terminal receptor to O2 such as Cytochrome C enzyme (25).

According to morphological and biochemical tests among collected samples 80 (28.6%) were bacterial growth or culture , while 200 (71.4%) were negative samples, bacterial growth or culture included 61(76.2%) gram negative (Gr -ve) and 19 (23.8%) were gram positive (Gr +ve), bacterial growth or culture were distributed as following, 50 (62.5%) samples *K. pneumonia*, 3 (3.8%) *Pseudomonas aeruginosa*, 5 (6.2%) *Acinetobacter baumanii*, 4 (5%) *Micrococcus,* 12 (15%) *Staphylococcus aureus,* 3 (3.8%) *Enterococcus cloacae* and 3 (3.8%) were *Aerococcus viridans.* negative samples distributed into 200 (66.6%) normal flora and 100 (33.4%) were contaminated samples. These outcomes were agreed with Guzek et al. (2022) (26) reported that during COVID-19 pandemic in Poland included 103 patients, of whom 23 (73.9%) were positive for K. pneumonia, in addition in the period of Arcari et al. (2021) (27) study, showed that Carbapenemase-producing Klebsiella pneumoniae were detected in 14/41 patients (34%) only followed by *P. aeruginosa*, *Micrococcus, Acinetobacter baumanii* and *S. aureus.* Overall, Alqahtani et al. (2022) (28) found that almost 70% of the co-infected with gram-negative organisms, K. pneumonia was the most frequently reported organism among all other bacterial isolates 57.69%.

While current data were non compatible with Stefanini et al. (2021) (29) showed that a total of 100 species, distributed among 33 genera, the most abundant species in covid-19 was Escherichia coli (420 isolates), followed by Klebsiella pneumoniae (n = 192), Pseudomonas aeruginosa (n = 187), Enterococcus faecalis (n = 184), Staphylococcus epidermidis (n = 175), and Staphylococcus aureus (n = 134). The observation of Acinetobacter baumannii/haemolyticus being more

abundant among the COVID-19 isolates is not surprising, as this species is known to cause ventilator-associated and bloodstream infections (30). This difference in outcomes may due to sample sizes in each study because the sample size increases, the confidence interval tends to become smaller, meaning that the research can have greater confidence in the reliability of the findings, in addition results possibly associated with the use of invasive devices widely used in the treatment of COVID-19 (ventilator and urinary catheters), may support the current dread of increase of nosocomial infections in COVID-19 pandemic and the use steroid that directly affect results of bacterial culture. In spite of that, most patients included in this study 200 (71.4%) of patients samples were negative in bacterial cultures. This may due to recent antibiotic use, presence of fastidious organisms such as nutritionally variant groups and inadequate samples in some cases. Another reasons for negativity may be that the patients had non-bacteremic illness or bacteria were present intermittently or at very low blood density. However, an additional explanation is that the causative microorganisms are delicate, fastidious, nonviable, slow growing at low densities, or uncultivable in culture medium. or when antimicrobial treatment has been started before blood collection, which may kill or inhibit pathogen growth. These diagnoses may therefore be missed and may be referred to as “false negatives.”

In this study patients age ranged between (41-50) and (51-60) were more frequent among all age groups, these information were in consistence with (Dergaa et al. (2022) (31). In severe infections the respiratory and urinary tracts are the most frequently involved systems which may be accompanied by severe sepsis. Bacteremia and sepsis are also associated with indwelling vascular catheters in the elderly who are admitted to the intensive care unit (ICU) (32, 33). While present outcomes were not agreed with other mentioned that it was does not suggest that the oldest individuals necessarily play the leading role in the spread of SARS-CoV-2 in the community, suggest that younger adults, particularly those aged under 35y often experience the highest cumulative rates of infection (34, 35). The differences in results among studies may belongs to long or shortness in a period of data collection in each study. Current results were in agreement with Papadopoulos et al., (2021) who showed that aimed to provide a review of the research literature, propose hypotheses, and therapies based on the potential link to COVID-19 in elderly men compared to women, aging, inflammation, severe acute respiratory syndrome (SARS) due to coronavirus infection, and COVID-19 disease state and outcomes was performed, the link between the immune system and male aging is well-established, as is the progressive decline in T levels with aging. In women, T levels drop before menopause and variably increase with advanced age. Elevated IL-6 is a characteristic biomarker of patients infected with COVID-19 and has been linked to the development of the acute respiratory distress syndrome (ARDS), these data suggest that low T levels may exacerbate the severity of COVID-19 infection in elderly men, T levels in aging hypogonadal males create a permissive environment for severe responses to COVID-19 infection or if the virus inhibits androgen formation. Given the preponderance of COVID-19 related mortality in elderly males, additional testing for gonadal function and treatment with T may be merited (36). In addition reports from China indicate that men accounted for 60% of COVID-19 patients (37). Moreover, 67% of patients admitted to the intensive care unit (ICU) were reported to be men (38). These data seem to indicate that there might be a gender predisposition to

COVID-19, with men predisposed to being more severely affected and older men accounting for most deaths (39). According to our knowledge all present research indicated the prevalence of men in covid-19 in comparison to women.

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