The Role of Procalcitonine in the Investigation of Secondary Bacterial Infections Associated with Sars-Cov and Determined Its Resistance to Antibiotics

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Abstract

110 samples (Blood, swab and sputum) were collected from the same persons from patients confirmed to infected with the emerging SARS-COV-2, for the period from November 2021 until March 2022. The age groups ranged 18-90 years old from both gender. The sputum and swab were cultivated on selective and differential media in the laboratory and stained by Gram stain. Some biochemical tests were done as confirmation diagnostic tests. There were 105(95.5%) samples yielded positive growth that includes 27 isolates (25.7%) are *Staphylococcus aureus*, 21 isolates (19.2%) are *Klebsiella pneumoniae*, two isolates (1.9%) are *Klebsiella oxytoca*, 16 isolates (15.2%) are *pseudomonas aeruginosa*, 9 isolates (8.5%) are *Haemophilus influenza*, and 8 isolates are (7.3%) of *Enterococcus feacilus* and 13 isolates of the genus Streptococcus 9 (8.2%) of which are *Streptococcus pneumoniae*, 4 isolates of (3.6%) of *Streptococcus pyogenes*, 5 isolates of (4.7%) of *Escherichia coli*, and 4 isolates of (3.8%) of *Moraxiella catarrhalis*. The sensitivity test of the isolated bacteria to 11 antibiotics were performed. The isolates varied for their response to the antibiotics used in the current study. And all isolates showed high resistance to some antibiotics, where all isolates showed 100% resistance towards Oxacillin. *S. aureas* was resistant to antibiotics Trimethoprin, Azethromycin, Oxacillin. *S.pneumoniae* was resistant to Tetracyclin, Oxacillin, Erythromycin, and *S. pyogenes* bacteria were resistant to Amoxicillin\Clavulanic acid, Tetracyclin, Oxacillin, Amikacin, Trimethoporacin, Eprothromycin, and *E. faecalis* was resistant to oxacillin. As for Gram-negative bacteria, the results showed that they were resistant to most antibiotics used in the current study. Procalcitonine test was used to detect and investigate secondary bacterial infections of SARS-CoV-2 patients in the serum of confirmed COVID-19 patients. The result showed procalcitonin was higher than the normal rate in the presence of bacteremia, which is an indication of the prognosis of a possible bacterial infection.

Keyword: respiratory tract, procalcitonine, secondary infections, SARS-CoV, antibiotics, bacteremia.

1. Introduction

The respiratory system supplies the body of living organisms with necessary and necessary oxygen to carry out its activities The body gets rid of carbon dioxide (Hsia et al, 2016). The respiratory system is divided into two parts: the upper respiratory system and the lower respiratory system. The upper part includes the nose, mouth, pharynx and larynx. This part is important and necessary for humans, because it remains open to be able to breathe and also It has a role in humidifying the air before it reaches the lungs (Gilroy et al, 2008), The oral cavity is considered more dangerous because it contains many microbes that move to the lungs and then to different areas of the human body (Mojon, 2002),Infectious respiratory diseases arise during the colonization of various microbes, including viruses and bacteria, as well as fungi in the areas of the mouth, larynx and pharynx (Korppi et al, 1989). The interaction occurs between the different forms, especially viruses and bacteria, and a wide range of disorders occurs, including tonsillitis, pharyngitis, bronchitis, and nasopharyngitis, which may lead to pneumonia (Simoes et al, 1999). Patients with a respiratory infection are at risk of developing a bacterial infection secondary leading to increased morbidity and mortality COVD-19 manifests with a rang of severity. The disease rang from no specific flu-like symptoms to life threatening pulmonary complication such as acute respiratory syndrome and organ failure. Secondary bacterial insomnia is a major factor for those infected with COVD-19 (Gosh et al, 2021). Staphylococcus aureus, Staphylococcus aures, Streptococcus pneumpnia, Neisseria meningitids, Haemophilus influenzae, and Klebsiella pneumonia. It is the most common bacteria isolated during secondary infection (Handel et al, 2009) where hospitals are a common source of pathogens that cause secondary infections, and this so-called pathogenic infection is acquired from an environment in which antibiotics are circulated, and as such many bacteria acquire Extensive resistance to a range of these antibiotics is due to misuse and overprescribing of antibiotics. Early diagnosis, diagnosis, and treatment in patients is essential because of the risk of permanent morbidity or death

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(Gupte and Chowdhry, 2002; Rennie, 2005). There is a definitive diagnostic criterion, by which bacteriuria is diagnosed, in which about 31% of patients have sepsis over the traditional methods followed (Bates et al, 1997). Procalcitonin PCT is a biomarker widely used to assess the risk of bacterial infection and disease progression, in patients with bacterial sepsis and suspected or confirmed lower respiratory tract infections, including community-acquired pneumonia, acute pneumonia, acute bronchitis and acute exacerbations. For COPD, procalcitonin can be a useful decision-making tool regarding antibiotic therapy. (Schuetz et al, 2018) The efficacy of procalcitonin as a diagnostic tool for lung and respiratory infections has already been demonstrated in several studies. There are also strong indications of its benefits in patients with COVID-19, but because the disease is new and the evidence is still developing, many studies show that Procalcitonine has significant predictive effects (Gregoriano et al, 2020).

2. Materials and Methods

1.samples Collection: (Blood, swab and sputum) were collected for the same persons from patients confirmed to infected with the emerging SARS-COV-2, who were diagnosed clinically and laboratory by doctor and specialized laboratory (males and females) and their ages ranged from (18-80 years). Blood samples were separated by centrifuges device to obtain the serum for the purpose of conducting the procalcitonine test supplied by the company (MYBioSource, USA), to detected and investigate the bacteria in blood by ELISA method.

2. Cultivation of samples: All samples were cultivated on selective and differentiated media. Morphological characteristics (colony size, color, edge, height) were examined. Gram stain was performed in order to observe their shapes and arrangement.

3. Biochemical Characteristics: A set of biochemical tests were conducted to diagnose the bacterial isolates under study (Forbes et al, 2005)

4-To determine antibiotic sensitivity of the isolates, disc diffusion method was used (Bauer and Kirby, 1966). Colony transferred to clean tube contain 4 ml of nutrient broth and incubated at 37 °C for 4-5 hours. Mueller Hinton agar plates (Himedia/ India) used as a standard medium for antibiotics test. The plates were incubated for 24h at 37°C and organisms were categorized as sensitive or resistant, based on the standards (Humphries et al, 2021). A total of 11 antibiotics used in this study: Lenvoflaxain (LEV), Azythromycin (AZM), Amoxilin/Clavulanic acid (AMC), Amikacin (AK), Tobramycin (TMB), Tetracyclin (TE), Trimethoprin (TMP), Ciprofloxain (CIP), Erythromycin (E), Gentamicin (CN), (Imipenem (IMP), Oxacillin (OX)/ Bioanalyse/ India).

5. Procalcitonine test: The procalcitonin level was estimated in the serum by using ELISA technique to prognoses the bacteremia by means of the procalcitonin level estimation kit supplied by the company.

3. Results and Discussion

Depending on the cultural characteristics of the microbes grown on the primary, selective and differential media, 105 (95.5%) samples produced a positive culture, while 8 (4.5%) did not show any bacterial growth. The lack of growth may be due to the fact that the infectious agents may belong to other organisms that cannot be diagnosed by routine diagnostic methods. Table (1): percentage of microbial growth

Table (1) shows that 27 (25.7%) Staphylococcus aureus isolates, 21 (20%) pneumoniae Klebsiella isolates, 2 (1.9%) oxytoca Klebsiella, 16 (15.2%) isolates from pseudomonas aeruginosa and 9 (8.6%) isolates from Haemophilus influenzae, 8 isolates (7.6%) of Enterococcus feacilus, 13 isolates of the genus Streptococcus, 9 (8.6%) of which Streptococcus pneumoniae, 4 isolates (3.8%) of Streptococcus pyogenes, 5 isolates (4.8%) of Escherichia coli, 4 isolates (3.8 %) of Moraxiella catarrhalis. The results showed that the percentage of secondary bacterial infections caused by negative bacteria reached positivity (55.2%) and (44.8%), and respectively, these results agreed with what was found (Marqabi, 1999), which showed that the percentage of respiratory infections caused by negative bacteria is higher than that caused by negative bacteria. Gram-positive bacteria, and the reason for this may be due to the natural presence of most of the gram-positive bacteria in the upper respiratory tract.

Table(1) Respresents the positive isolates, their number and percentage.

NO.	Sample	Number	Percentage%
1	Escherichia coli	5	4.8%
2	Haemophilus influenzae	9	8.6%
3	Klebsiella pneumoniae	21	20%
4	Klebsiella oxytoca	2	1.9%
5	Moraxiella catarrhalis	4	3.8%
6	Pseudomonas aeruginosa	16	15.2%
7	Staphylococcus aureus	27	25.7%
8	Streptococcus pneumoniae	9	8.6%
9	Streptococcus pyogenes	4	3.8%
10	Enterococcus faecalis	8	7.6%
	Total	105	100

Acording to Clinical and Laboratory Standards Institute (Humphries et al, 2021), antibiotic sensitivity has been determined. The result in table (2) the resistance of isolated bacteria to 11 antibiotics, the isolates varied for their response to antibiotics. And isolates showed high resistance to some antibiotic, where all isolates showed 100% resistance towards Oxacillin, the result are in agreement with (Amin, 2021). Antibiotic resistance has become a serious threat to human health, and this may be due to the widespread use of antibiotics, as well as the lack of awareness of community members. (Al-Sanafi, 2010).

Table (2) the resistance of bacterial isolates to antibiotics												
Bacterial Isolation	No	AMC	AK	AZM	CIP	CN	E	LEV	IMP	OX	TMP	TE
		N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N (%)	N(%)
S. aureus	27	(100) 27	(29.6) 8	(100) 27	(81.5) 22	(55.6) 15	(100) 27	(77.8) 21	(%) 0	(100) 27	(100) 27	(25.9) 7
S. pneumoniae	9	(100) 9	(55.6) 5	(66.7) 6	(44.4) 4	(22.2) 2	(33.3) 3	(55.6) 5	(55.6) 5	(100) 9	(66.7) 6	(100) 9
S. pyogenes	4	(100) 4	(100) 4	(75) 3	(100) 4	(%) 0	(100) 4	(100) 4	(50) 2	(100) 4	(100) 4	(100) 4
E. faecalis	8	(100) 8	(25) 2	(75) 6	(50) 4	(25) 2	(87.5) 7	(50) 4	(50) 4	(100) 8	(75) 6	(75) 6
K. pneumoniae	21	(100) 21	(9.5) 2	(85.7) 18	(85.7) 18	(0) 0	(100) 21	(85.7) 18	(0) 0	(100) 21	(90.5) 19	(85.7) 18
K. oxytoca	2	(100) 2	(50) 1	(100) 2	(100) 2	(0) 0	(100) 2	(100) 2	(100) 2	(100) 2	(50) 1	(50) 1
H. influenzae	9	(100) 9	(77.8) 7	(100) 9	(100) 9	(66.7) 6	(100) 9	(88.9) 8	(33.3) 3	(100) 9	(100) 9	(100) 9
P. aeruginosae	16	(100) 16	(93.8) 15	(75) 12	(100) 16	(87.5) 14	(100) 16	(100) 16	(100) 16	(100) 16	(87.5) 14	(93.8) 15
E. coli	5	(100) 5	(80) 4	(0) 0	(40) 2	(40) 2	(100) 5	(80) 4	(20) 1	(100) 5	(100) 5	(100) 5
M. catarrhalis	4	(100) 4	(50) 2	(75) 3	(50) 2	(25) 1	(75) 3	(100) 4	(25) 1	(100) 4	(75) 3	(25) 1

Screening for sepsis with Procalcitonine

The results in the table (3) showed that the values of procalcitonin were high in people infected with some bacterial species (S. aureus, S. pyogenes, P. aeroginosa, S. pneumoniae K. pneumonia, E. faecalis, E. coli and H. influenza) there are no significant difference with respect to bacterial species, when comparing of values of procalcitonin with the normal values, we notice an increase in procalcitonin, which is an indication of the prognosis of a possible bacterial infection. These results are for archiving with a study (Hu et al, 2020).

Table (3) evaluation of procalcitonin in patients with secondary infections with SARS-Cov-2.								
normal values	S. pneumoniae	H. influenzae	E. coli	E. faecalis	K. pneumoniae	P. aeroginosa	S. aureus	S. pyogenes
425-325 ml/Pg	149±755	743±77	644±248	161±699	161±711	732±221	127.4±684	0±742
	a	a	a	a	a	a	a	a
*Similar letters mean there are potential difference in the level P< 0:05								

Bacterial infections associated with COVID-19 and causing bacteremia as shown in tablet (4) is the number of confirmed bacterial infections in bacteremia and those that do not cause bacteremia

Table 4: number and types of bacteria that cause bacteremia			
Types of bacteria	Numbers	PCT +	PCT -
S. aureas	27	10	17
S. penumoniae	9	7	2
S. pyogenes	4	1	3
E. feacalis	8	5	3
K. penumoniae	21	16	5
K. oxytoca	2	0	2
E. coli	5	3	2
P. aeroginosa	16	12	4
H. influenzae	9	5	4
M. catarrhalis	4	0	4
Total	105	(%56.2) 59	(%43.8) 46

The results showed that the positive bacteria were 23 with 39% while the negative bacteria were 36 with 61% of endotoxin is one of the strong inducers of PCT and it was found (Dandona et al, 1994) a significant increase in Procalcitonine after endotoxin injection in normal subjects. Gram-negative bacterial species are responsible for about 24% of health care bacteremia cases and about 45% of community-acquired bacteremia (Schuetz et al, 2018). The most common types are P. aeroginosae, K. pneumoniae, and E. coli (Deen et al, 2012). Gram-positive, and its effect on bacteremia, the percentage of staphylococcus aureus, streptococcus and enterococci, staphylococci is the main cause of health care-associated bacteremia and also a cause of acquired bacteremia (Biedenbach and Jones, 2004).

4. Conclusions

It can be concluded from this study that it is possible to predict the bacterial infections associated with SARS-CoV for the purpose of prescribing the appropriate drugs for it.

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