



Detection of Bacterial causes of diarrhea in patients with SARS-CoV-2 in Samarra city

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<https://doi.org/10.25130/tjps.v27i4.28>

ARTICLE INFO.

Article history:

-Received: 4 / 1 / 2022

-Accepted: 19 / 4 / 2022

-Available online: / / 2022

Keywords: COVID-19, bacterial Co-infection, antibiotic sensitivity pattern

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1. Introduction

Covid-19 pandemic has spread around the world according to the statement of the World Health Organization (WHO) last December 2019, with the first infections recorded from China and transmitted to the rest of the world, a global emergency was imposed all over the world [1], as well as in Iraq, which added a serious challenges on the health and economic sectors in the country, especially with conflicting data about the source of infection and ways to control the virus [2]. Bacterial secondary infection is a common complication of viral infection with increased morbidity and mortality concomitantly with an increased burden on health care resources [3]. Several cases of bacterial infections associated with COVID-19 have been reported despite declining rates of influenza and other viral diseases[4], according to studies have shown an increase in the number of common opportunistic pathogens such as *Enterococcus faecalis* and family *Enterobacteriaceae* that includes *Escherichia coli* and *Klebsiella pneumoniae* in critically ill COVID-19 patients with poor prognosis [5]. Because the focus and attention on the treatment and control of COVID-19 has neglected the common bacterial infection in COVID-19 patients [6]. Rural areas, refugee camps and slums are the epicenter of many bacterial diseases, and bacterial diarrhea is the highest prevalence due to poor health services provided [7]. It is also a suitable

ABSTRACT

The current study aimed to detection of bacterial causes of diarrhea in patients with SARS-CoV-2 in Samarra city, for this purpose, 71 stool samples collected from SARS-CoV-2 patients with diarrhea, bacterial culture techniques, biochemical tests and vitik2 were used. The results showed that out of 71 sample, 45 Gram-negative isolates (63.38%) and 26 Gram-positive isolates (36.62%). Main bacteria spp. Were *Escherichia coli*, *Klebsiella pneumoniae*, *proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Enterococcus faecalis*

environment for the spread of antibiotic-resistant bacterial strains due to the uncontrolled use of antibiotics

2.1 Materials and methods

In this study, (71) stool samples were collected from patients infected with covid-19 from Samarra Hospital-quarantine unit and different laboratories and health centers s in Samarra city, aged (15-70) years suffering from diarrhea. The period of sample collection was between November 2020 and April 2021. These samples were transferred into sterile plastic tubes containing a nutrient broth as a nutrient and transport medium then transported to the Microbiology Laboratory in Iraq. College of Applied Sciences - University of Samarra to be examined.

2.2 Isolation and identification of bacterial species by traditional methods

Diagnosis of isolates focused on morphological characteristics and conventional biochemical tests. Stool samples were examined microscopically in the laboratories of Samarra General Hospital and the laboratories of health centers within the city, where the samples were collected, and then transferred to the laboratories of the College of Applied Sciences - University of Samarra by using nutrient agar broth as a transport medium.

The bacteria were isolated from the samples by cultivation on nutrient broth culture medium at 37C

for 24 hours, then after dilution of samples with normal saline sub-culturing was done on nutrient agar, blood agar and were identified by performing Gram staining, differential bacterial growth and selective media were cultured such as MacConkey agar, mannitol salt agar, S.S agar, and XLD, and finally the isolates was diagnosed with Vitek-2 Compact system (bio-Merieux) [9].

2.3 Antibiotic Susceptibility Testing

Antimicrobial sensitivity patterns of the stool samples were performed by using Kirby Bauer disc diffusion method on Mueller Hinton agar [10].

As recommended by the clinical and laboratory standards institute (CLSI)[11].

Antibiotics which were tested include:

Ampicillin (25µg), Amoxicillin (25 µg),
Azithromycin(15 µg), Ciprofloxacin(10 µg),
Levofloxacin(5 µg), Ceftriaxone(10 µg),
Gentamicin(10 µg), Tetracyclin(10 µg),
Vancomycin(30 µg), Rifampicin(5 µg).

3. Results

3.1 bacterial Isolation

A total of 71 stool samples were collected from patients infected with COVID-19 accompanied with diarrhea with throughout the study period to detect on 45 Gram-negative isolates (63.38%) including the species:

Escherichia coli 17 isolates, *Klebsiella pneumoniae* 5 isolates,

Klebsiella oxytoca 8 isolates, *proteus mirabilis* 7 isolates, *Pseudomonas. aeruginosa* 6 isolates, *Salmonella. typhimurium* 2 isolates, and 26 Gram-positive isolates (36.62%) including the species:

Staphylococcus aureus 9 isolates, *Staphylococcus epidermidis* 9 isolates, and 8 isolates of *Enterococcus faecalis*. These results agreed with the results of study in Al-Bahrain kingdom [12].

3.2 Antibiotic Resistance Patterns

The results in table(1) revealed that (47.05%) of *E.coli* isolates were resistant to azithromycin, Ampicillin,gentamicin and ceftriaxone, the resistance mechanism of *E.coli* as well as other *Enterobacteriaceae* members can be explained by using chromosomal efflux pumps as intrinsic and acquired resistance towards azithromycin and other macrolides [15], (82.35%) amoxicillin, (94.11%) to tetracycline and rifampicin and 100% resistance to Vancomycin, while (23.52%) of the isolates was resistant to levofloxacin and ciprofloxacin. These results agreed with [13] that recorded resistance of *E.coli* (27.27%) towards levofloxacin,(66.66%) to ciprofloxacin, also our results agreed with[14] that recorded (100%) resistance to vancomycin, (81.82%) to ceftriaxone, (54.55%) to ciprofloxacin. *E.coli* as other *Enterobacteriaceae* members resist tetracycline antibiotics by blocking the uptake of drugs into cells due to the efficiency and activity of the efflux pump system, which works to reduce the permeability of the membrane resulting from mutations in chromosomes as well as inhibiting the activity of the

drug by preventing its association with ribosomes in the cytoplasm of the cell [16]. Vancomycin resistance in *Enterobacteriaceae* and other gram -negative bacteria is an intrinsic resistance due to its complex structure which blocks its penetration through the outer membrane of, and exerts limited bactericidal effect on gram-negative bacteria[17].

The whole isolates of *K.pneumoniae* were resistant to amoxicillin, ampicillin,vancomycin and rifampicin (100%), (80%) to azithromycin, ceftriaxone and tetracycline while resistance level was decreased to (20%) to ciprofloxacin, levofloxacin and gentamicin. These results agreed with [18] that recorded (90%) resistance of *K.pneumoniae* towards tetracycline, (25%) to ceftriaxone, (90%) to ampicillin to (50%) gentamicin, (0%) to ciprofloxacin, while our results disagreed with [19] that recorded (11.11%) resistance towards ceftriaxone and (57.41%). *K pneumoniae* resist aminoglycosides by aminoglycosides-modifying enzymes (AMEs) genes, using the efflux pump that modify the cell permeability [20]

The whole isolates of *K.oxytoca* were (100%) resistance to vancomycin and rifampicin, (87.5%) to tetracycline, (75%) to amoxicillin, azithromycin, (50%) to ceftriaxone, (12.5%) to levofloxacin and gentamicin, (50%) to ampicillin, while whole the isolates was sensitive to ciprofloxacin. These isolates agreed with [21] that founded resistance of *K. oxytoca* towards gentamicin (35.8%) and tetracycline (37.5%). Resistant isolates of *K. oxytoca* regarding biofilm formation [22].

With (57.14%) resistance of *proteus mirabilis* isolates to amoxicillin, (42.85%) to gentamicin, ampicillin, (85.5%) to Azithromycin, tetracycline, a low resistance to fluoroquinolones group antibiotics levofloxacin (0%), ceftriaxone and ciprofloxacin (28.57%) without any antibacterial activity of rifampicin and vancomycin. Our results agreed with [23] that founded (97.5%) resistance percentage to tetracycline and azithromycin, while 19.5% resistance to levofloxacin. In other study by[24] showed (77.3%) to ciprofloxacin, (77.9%) to gentamicin And(58%) to tetracycline. *Proteus mirabilis* resist vancomycin due to its intrinsic resistance as a gram negative bacteria has a low permeability against the hydrophilic, large molecular weight vancomycin [25]. *P.mirabilis* resist rifampicin by chromosomal mutation which prevents binding in RNA polymerase [26]. *P .mirabilis* resist macrolides and tetracycline through the Major Facilitator Super family (MFS) efflux pump [27], *P.mirabilis* resist β -lactams antibiotics through producing Extended-Spectrum-Beta-Lactams enzymes (ESBLs) that destruct the β -lactam ring then hydrolysis the drug [28].

The six isolates of *Pseudomonas aeruginosa* was resistant (100%) to rifampicin, vancomycin, and tetracycline, (83.33%) to ampicillin, (50%) to amoxicillin, azithromycin, and gentamycin, (33.33%) to levofloxacin, (16.66%) to ceftriaxone and ciprofloxacin. Our results disagreed with [29] that

recorded 100% resistance towards ceftriaxone, amoxicillin and ampicillin. *P.aeruginosa* possesses a low permeability outer cell membrane as intrinsic resistance mechanism in addition to the transmembrane efflux pumps [30], biofilm formation also possessing resistance -plasmid and (ESBLs)[31].

The tow isolates of *Salmonella typhimurium* was totally resistant to rifampicin, vancomycin and ampicillin, (50%) to tetracycline, gentamycin, amoxicillin and ceftriaxone, while the isolates were totally sensitive to azithromycin, levofloxacin, and ciprofloxacin with (0%) resistance. Our results disagreed with [32] that recorded (100%) resistance to ampicillin, (53.6%) resistance toward ciprofloxacin, (17.9%) to levofloxacin. Extrusion of antibiotics through efflux pumps is one of the mechanisms resistant that obtained by *S.typhi* [33]

Staphylococcus aureus isolates were (88.88%) resistant to gentamicin, (77.77%) to, rifampicin, (66.66%) to Vancomycin and ciprofloxacin, (44.44%) to tetracycline, and azithromycin, (33.33%) to amoxicillin, ceftriaxone, and ampicillin, (11.11%) to levofloxacin. Our results agreed with [34] that recorded (78.95%) resistance toward vancomycin and ciprofloxacin, (84.21%) rifampicin, (100%) to gentamicin, also agreed with [35] that recorded the resistance ratios as: (85%, 77.9%,) 77.3%,68.2%, 58%,31.3%) toward amoxicillin, gentamicin, ciprofloxacin, tetracycline, azithromycin and ampicillin.

S.aureus developed resistance to fluoroquinolones through mutagenesis [36], *S.aureus* resist aminoglycosides through decreasing outer cell membrane permeability which decreasing drug intake[37], *S. aureus* develop resistance to vancomycin through two categories of mechanisms including: Vancomycin mediated resistance which is carried by transposon and resistance due to thickened cell wall[38].

Enterococcus faecalis isolates were totally resistant (100%) to tetracycline, (87.5%) resistance to vancomycin, and gentamicin, (75%) to azithromycin and rifampicin, (62.5%) to ampicillin, and a mild resistant (37.5%) to amoxicillin and ceftriaxone, (25%) to ciprofloxacin, (12.5%) to levofloxacin. Our results disagreed with[39] that recorded (92.86%) resistance ratio toward rifampicin, (60.71%) resistance toward ciprofloxacin, (57.14%) toward tetracycline, (46.43%) resistance toward Vancomycin. *Enterococcus faecalis* possess intrinsic resistance to β -lactam antibiotics due to mutation or over production of penicillin-binding proteins (PBPs) and releasing of β -lactamases enzymes [40] developed resistance mechanism through inducing mutation in β - subunit of RNA polymerase[41]. *Enterococcus faecalis* Resist vancomycin by gene mutation on

Plasmid or transposons that enable bacteria to be eliminated

From the effect of antibiotics in interrupting the chain of cell wall formation process[42].

Table 1: Antibiotic resistance percentage of bacterial isolates

Isolated bacteria	Types of antibiotics									
	AM	AX	AZM	CN	TE	CRO	LEV	CIP	VA	RA
<i>E.coli</i>	47.05 %	82.35 %	47.05 %	47.05 %	94.11 %	47.05 %	23.52 %	66.66 %	100%	94.11 %
<i>K.pneumoniae</i>	100%	100%	80%	20%	80%	80%	20%	20%	100%	100%
<i>K.oxytoca</i>	50%	75%	75%	12.5%	87.5%	50%	12.5%	0%	100%	100%
<i>Salmonella.typhi</i>	100%	50%	0%	50%	50%	50%	0%	0%	100%	100%
<i>P.aeruginosa</i>	83.33 %	50%	50%	50%	100%	16.66 %	33.33 %	16.66 %	100%	100%
<i>Proteus.mirabilis</i>	42.85 %	57.14 %	85.5%	42.85 %	85.5%	28.57 %	0%	28.57 %	100%	100%
<i>S.aureus</i>	33.33 %	33.33 %	44.44 %	88.88 %	44.44 %	33.33 %	11.11 %	66.66 %	66.66 %	77.77 %
<i>Enterococcus.faecalis</i>	62.5%	37.5%	75%	75%	100%	37.5%	12.5%	25%	87.5%	75%

Discussion

The emergence of the disease (SARS-CoV-2) is a real threat to global health that deserves directing all efforts towards it, an attempt to find out its causes to control it and reduce the risks of exposure to it. The secondary bacterial infection adds another burden that requires quick action because of the additional risks it causes to public health, poor diagnosis and attrition. Health care resources and the emergence of more antibiotic-resistant bacterial strains. The presence of bacterial co-infection in the gastrointestinal tract of

patients with COVID-19 has not been studied, especially in the Republic of Iraq with many displaced individuals living in remote areas. It is difficult to record health data and control the spread of infection and know whether the bacterial infection was community acquired or hospital-acquired. Our study identified the etiology of common bacterial infections through microscopic and cultural examinations of stool samples from diarrhea patients collected in Samarra city, which was *Escherichia coli* isolated by the study, followed by

Klebsiella oxytoca, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Salmonella typhimurium* While the Gram-positive bacteria isolated during the study were *Staphylococcus aureus* *Staphylococcus epidermidis* and *Enterococcus faecalis*

Conclusion

Common bacterial infection can occur before a patient is admitted to hospital (community acquired) or it can occur as a secondary infection due to opportunistic bacteria (hospital acquired). Bacterial infection, usually associated with respiratory viral

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infection, is one of the main causes of difficulty in diagnosis and increased morbidity and mortality, especially in the elderly and immunocompromised. Increasing pressure on the healthcare sector as well as the emergence of more bacterial strains that are resistant to antibiotics. To study the presence and characteristics of common gastrointestinal bacterial infection in patients with COVID-19, in Samarra, Iraq, efforts must be intensified to find out the causes and try to control the disease and the common bacterial infection.

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التحري عن المسببات البكتيرية للإسهال لمرضى كوفيد-19 في مدينة سامراء

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المخلص

هدفت الدراسة الحالية إلى التحري عن المسببات البكتيرية للإسهال لدى مرضى كوفيد-19 في مدينة سامراء، ولهذا الغرض تم جمع 71 عينة براز من المرضى الراقدين في مستشفى سامراء العام _ وحدة العزل لمرضى كوفيد-19 بمراحل مختلفة من المرض ولمختلف الجنسين ممن يعانون من الإسهال، خلال الفترة تشرين الثاني 2020-نيسان 2021 استعملت تقنيات الاستزراع البكتيري والاختبارات الكيموحيوية وتقنية جهاز الفايكك2. أظهرت النتائج أنه من أصل 71 عينة ، 45 عذلة سالبة الغرام (63.38%) و 26 عذلة موجبة الغرام (36.62%).

كان النوع الرئيسي المسبب للإسهال هو *Escherichia coli* ثم تلتها الأنواع البكتيرية:

Klebsiella pneumoniae, *Klebsiella oxytoca*, *Proteus mirabilis* *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*.