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Antibiotic Resistance Profile of *Escherichia coli* Isolated from Stool Samples of COVID-19 Patients

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Abstract

Background: Antimicrobial resistance (AMR) poses a serious worldwide threat besides causing several concerns for the health system. *E. coli* has evolved to be capable of acquiring antibiotic resistance characteristics from other bacteria in its environment and of being easily transferred via fecal-oral route. However, this concern has not received enough attention during the last COVID-19 pandemic. *Aim:* To evaluate the antimicrobial profile of fecal *E. coli* isolated from COVID-19 patients. *Subjects and methods:* This study was conducted on 180 patients divided into two groups: the group of COVID-19 patients and the group of non-COVID-19 patients (90 each). Fecal samples were subjected to microbiology procedures for isolation and identification of *E. coli* strains. The identified strains were subjected to antimicrobial susceptibility testing using Kirby-Beuer test (disc diffusion method) and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. *Results:* The study found that a substantial proportion (82.2%) of patients with COVID-19 received azithromycin. Furthermore, the COVID-19 group demonstrated a significantly higher resistance to ertapenem compared to the non-COVID-19 group (8.9% versus 0%). Notably, *E. coli* isolates from COVID-19 patients exhibited a considerably higher multi-drug resistance (MDR) profile than those from non-COVID-19 patients (55.3% versus 44.7%). C*onclusion: E. coli* isolated from fecal samples of COVID-19 patients showed resistance to many tested antibiotics . Although, they showed significant resistance to ertapenem, the most efficient drugs against *E. coli* were the carbapenems. MDR *E. coli* was observed in both groups signifying community-acquired resistance. However, significant MDR pattern was detected among COVID-19 patients.

Keywords: Pandemic; COVID-19 patient microbiota; Fecal *E.coli*; Multidrug resistance; Gram-negative bacteria.

Introduction

Antimicrobial resistance (AMR) is a serious global health concern, with predictions suggesting it could lead to 10 million deaths by 2050. The prevalence of AMR varies significantly across different regions.(1) High-income countries (HICs) also face significant AMR challenges. For example, the United States reports approximately 2.8 million cases of antibiotic-resistant infections annually.(2) In contrast, lower-middle-income countries (LMICs) struggle to accurately assess the extent of AMR due to limited data. Data on AMR is unavailable for around 42.6% of African countries.(3)

During the COVID-19 pandemic, the widespread use of antibiotics, including drugs like azithromycin, both for treating bacterial infections and as an immune system modulator, was associated with an increase in antibiotic resistance. Systematic reviews revealed a low occurrence of bacterial coinfections and

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superinfections in COVID-19 patients, despite the excessive use of antibiotics.[4,5]
In many instances, the decision to treat COVID-19 patients with antibiotics for bacterial infections lacked solid evidence. These decisions were often based on the severity of the patient's condition or the clinician's prior experience, rather than on established criteria for co-infections or superinfections.[6]
Escherichia coli (*E. coli*), extensively studied worldwide, serves as a model organism for understanding antibiotic resistance. Its ability to spread resistance genes to other bacteria and contribute to the rise of multidrug-resistant (MDR) infections is a major concern. [7,8] This is facilitated by its easy transmission between humans and from animals to humans through the fecal-oral route, making it a significant driver of the global antibiotic resistance crisis. [9]
The rapid bacterial evolution under selective antibiotic pressure is one of the most significant causes that have contributed to the present AMR issue, since a persistent contact between bacteria and any received antibiotic is considered as a vital component for the MDR strains increase. [10]
Regrettably, the global response to this catastrophe has been insufficient despite the global rise in infections brought on by MDR bacteria. Individuals continue to overuse antibiotics and have even intensified this use over time. Analyzing global antibiotic sales statistics, it was shown that, from 2000 to 2015, the rate of antibiotic consumption worldwide grew by 39%, from daily doses/1000 individuals of 11.3 per day to 15.7. [10,11]
According to Klein et al., [11] LMIC account for the majority of the world's use of antibiotics and also have the greatest rates of infections caused by MDR bacteria. Usage of last-resort antibiotics like colistin and carbapenems is also increasing consistently with the emergence of resistant *E. coli* to these medications.[12]

Subjects and Methods

This study investigated the resistance patterns of *E.coli* isolated from stool samples collected from 90 COVID-19 patients and 90 non-COVID-19 patients.
Study Design and Setting: This comparative cross-sectional study was carried out during a period of eighteen month, from February 2021 through September 2022. The patients included in this study were recruited from a private diagnostic laboratory in Alexandria, where they had visited for routine health check-ups and diagnostic evaluations.

Study Population

Inclusion criteria:

-Non hospitalized COVID-19 patients with confirmed reverse transcription polymerase chain reaction (RT-PCR) result and / or clinical or radiological findings, who received antibiotic therapy during the duration of their COVID-19 infection.
-Non-COVID- 19 patients who received antibiotic therapy in the last six months before the study.

Exclusion criteria:

-COVID-19 patients with associated other diseases that required antibiotic treatment.
-Patients less than 18 years old.
-Non COVID-19 patients with history of COVID-19 or in contact with positive cases of COVID-19.

Sample size

Based on a previous study by Shakya et al.[13], a minimum required sample size of 90 stool samples of COVID-19 patients as well as 90 stool samples of non-COVID-19 patients achieve 80% power to estimate the proportion difference of antibiotic resistance of *E. coli* isolated from stool samples between the two groups. Sample size was estimated based on an average antibiotic resistance prevalence rate of 33% based on another published study from healthy subjects. An increase of 20% in antibiotic resistance pattern among COVID-19 patients was anticipated. Calculation was performed by comparing two binomial distributions at 0.05 level of significance. Sample size was calculated using MedCalc software.

Collection of samples

Stool samples were collected in sterile plastic cups and transported to the microbiology laboratory at the High Institute of Public Health, Alexandria University within 2 hours in an ice box. For each patient enrolled in this study, a comprehensive sheet was filled out, containing information such as sampling date, time of collection, name, age, sex, type and duration of antibiotics intake, and other relevant clinical data. Consecutive samples were collected till reaching the required sample size.

Isolation and Identification procedures:

All stool samples were directly plated using sterile loop on MacConkey's agar plates. Plates were aerobically incubated overnight at 37C°. Suspected isolated *E. coli* colonies were subcultured on blood agar plate for confirmation using standard microbiological procedures.(14,15) These procedures included macroscopic, microscopic and biochemical identification as shown in table (1).

Table (1): Biochemical tests for *E. coli*.

E. coli	*Results*
Triple sugar iron	
Slant/butt	Acidic/Acidic
H_2S	Negative
Gas	Positive
Indole production	Positive
MR	Positive
VP	Negative
Citrate utilization	Negative
Urease	Negative
Motility	Positive

Macroscopically, they showed bright pink lactose fermenting colonies on macConkey agar plates and non hemolytic non pigmented greyish convex smooth colonies on blood agar plates. *E.coli* appeared as rod shaped Gram negative bacteria with no special arrangement under the microscope.

All the identified *E. coli* isolates were subjected to antimicrobial susceptibility testing on Mueller Hinton agar (MHA) plates using disc diffusion method according to the Clinical Laboratory Standards Institute guidelines (CLSI) as illustrated in table (2). (16)

Table (2): Interpretive categories and zone diameters break points for Enterobacterales					
Test/Report Group	Antimicrobial agent	Disc content	Zone Diameter Interpretive Criteria (nearest whole mm)		
			Susceptible (S)	Intermediate (I)	Resistant (R)
Penicillins					
A	Ampicillin	10 µg	≥17	14-16	≤13
β-Lactam/β-lactamase inhibitor combinations					
B	Amoxicillin-clavulana	20/10 µg	≥18	14-17	≤13
Cephems (parenteral)					
B	Ceftriaxone	30 µg	≥23	20-22	≤19
A	Cefazolin	30 µg	≥23	20-22	≤19
B	Cefepime	30 µg	≥25	19-24	≤18
Carbapenems					
B	Ertapenem	10 µg	≥22	19-21	≤18
Aminoglycosides					
A	Gentamycin	10 µg	≥15	13-14	≤12
Quinolones and fluoroquinolones					
B	Cirpofloxacin	5 µg	≥26	22-25	≤21
Folate pathway antagonists					
B	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	11-15	≤10
Tetracyclines					
O	Doxycycline	30 µg	≥14	11-13	≤10
Monobactams					
C	Aztreonam	30 µg	≥21	18-20	≤17

Ethical considerations

An informed written consent was obtained from all participants after explanation of the purpose of the study. The study was approved by the Ethics Committee at the High Institute of Public Health (HIPH), Alexandria University.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 27.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean and standard deviation. Significance of the obtained results was judged at the 5% level ($p \leq 0.05$). [17] The used tests were:

1- Student t-test independent sample: for testing significance between parametric (≥30) quantitative variables in two studied groups.

2- Pearson's Chi-square test ($\chi 2$) **:** for categorical variables when ≤20% of cells with 5 value or less, to compare between different groups.

3- Fisher's Exact test : for testing significance between categorical variables when ≥20% of cells with 5 value or less.

Results

Table (3) shows distribution of the studied populations according to age and sex characteristics in relation to groups. In COVID-19 group, the age ranged from 18 to 79 years with mean value 43.81 ± 16.03 years, while in non-COVID-19 group, the age ranged from 18 to 77 years with the mean of 41.52 ± 16.80. There was no statistical significant difference between the two groups concerning age (t=0.935 and P = 0.351).

In COVID-19 group; males were 58.9% and females were 41.1%. While in non-COVID-19 group males were 53.3% and females were 46.7%. There was no statistical significant difference between the two groups concerning sex (χ2=0.564 and P=0.453).

Table (4) shows that the most affected age group was 30 to < 45 years by a percentage of 34.4% followed by the age group less than 30 years by 31.1%, then group 45 to < 60 years by 22.2% and the least was 60 or more years by 12.2%.

Table (3): Age and sex profile of the 180 COVID-19 and non-COVID-19 patients.

Age and Sex	COVID-19 n=90		Non COVID n=90		Analytic test	P value
Age (years)						
Range	18-79		18-77		t= 0.935	0.351
Mean ± S.D.	43.81 ± 16.03		41.52 ± 16.80			
Sex	N	%	N	%		
Male n=101	53	58.9	48	53.3	χ^2 =0.564	0.453
Female n=79	37	41.1	42	46.7		

t= Student t test, χ2= Pearson Chi-Square test, S.D.= standard deviation.
P value is considered significant if ≤0.05

Table (4): Age and sex distribution of the 90 COVID-19 patients.

Age group (years)	Sex (N=90)			Percent
	Male	Females	Total	
Less than 30	16	12	28	31.1%
30 to <45	20	11	31	34.4%
45 to <60	11	9	20	22.2%
60 or more	6	5	11	12.2%
Total number in males and females	53	37	90	
Total percent in males and females	58.9%	41.1%	100%	

Table (5) shows that concerning the the 90 COVID-19 patients, azithromycin was significantly used by 82.2% of cases (P≤0.001), followed by amoxicillin-clavulanic, ceftriaxone, ampicillin and doxycycline (42.2%, 12.2%, 7.8% and 7.8% respectively). The least to be used was ciprofloxacin by 3.3% of cases.

While regarding antibiotic usage in non-COVID-19 group, metronidazols were used by 63.32% of cases, followed by macrolides (53.32%), penicillins (44.4%), and the least used were 3rd generation cephalosporins (27.77%).

Table (6) shows the resistance profile of *E. coli* strains isolated from both COVID 19 and non-COVID 19 patients. *E. coli* strains from COVID-19 group were resistant to cefazolin, ampicillin, amoxicillin-clavulanic acid, cefepime, doxycyline and trimethoprim-sulfamethoxazol (91.1%, 61.1%, 38.9%, 35.6%, 32.2%, and 31.1% respectively). Nearly a similar pattern was obtained from *E. coli* strains from non - COVID-19 group for the same antibiotics (90%,73.3%, 52.2%, 40%, 40%, 32.2% respectively).
Significantly higher resistance to ertapenem was observed in COVID group versus non-COVID group (8.9% vs 0%) with P value equals 0.007. Significant higher resistance was observed in non-COVID group considering gentamicin (24.4% vs 7.8%, P value equals 0.004), ciprofloxacin (30% vs 15.6%, P value equals 0.032) and aztreonam (36.7% vs 21.1%, P value equals 0.032).

Table (7) illustrates that *E. coli* isolates from both COVID-19 and non-COVID-19 patients showed MDR profile (55.3% and 44.7% respectively) with a significantly higher percent of MDR among COVID -19 patients isolates (P=0.037).

Table (5): Distribution of antibiotics intake by the 180 COVID-19 and non-COVID-19 patients.

Antibiotic	COVID-19n=90		Non-COVID-19 n=90		Analytic test	P value
	N	%	N	%		
Macrolides						
Azithromycin	74	82.22	8	8.88	χ^2=97.57	≤0.001*
Clarithromycin	-	-	40	44.44	-	-
Penicillins						
Amoxicillin-Clavulanic acid	38	42.22	40	44.44	χ^2= 0.09	0.764
Ampicillin	7	7.77	-	-	-	-
3rd generation cephalosporins						
Ceftriaxone	11	12.22	18	20	χ^2=2.014	0.155
Cefotaxime	-	-	7	7.77	-	-
Quinolones						
Ciprofloxacine	3	3.33	-	-	-	-
Tetracyclines						
Doxycycline	7	7.77	-	-	-	-
Metronidazoles						
Metronidazole	-	-	57	63.32	-	-

χ2= Pearson Chi-Square test.
P value is considered significant if ≤0.05.
P value is considered highly significant if ≤0.005
Some patients take more than one antibiotic

Table (6): Antibiotic resistance profile of *E-coli* strains isolated from the 180 stool samples of the 180 COVID-19 and non-COVID-19 studied patients.

Mode of action	Antibiotic class	Antibiotic name	Disc content	Resistance profile		Test	P value
				COVID-19	Non-COVID-19		
Inhibition of cell wall synthesis	Penicillins	Ampicillin (AMP)	10 µg	55 (61.1%)	66 (73.3%)	χ2=3.051	0.112
		Amoxicillin clavulanic (AMC)	20/10 µg	35 (38.9%)	47 (52.2%)	Fissure Exact = 3.921	0.099
	Cephalosporins	Ceftriaxone (CRO)	30 µg	26 (28.9%)	37 (41.1%)	Fissure Exact =3.700	0.118
		Cefazolin (CFZ)	30 µg	82 (91.1%)	81 (90%)	Fissure Exact = 2.474	0.591
		Cefepime (FEP)	30 µg	32 (35.6%)	36 (40%)	χ2=0.378	0.645
	Carbapenems	Ertapenem (ETP)	10 µg	8 (8.9%)	0 (0%)	Fissure Exact =11.463	0.007*
	Monobactams	Aztreonam (AZT)	30 µg	19 (21.1%)	33 (36.7%)	χ2=5.300	0.032*
Inhibition of protein synthesis	Aminoglycosides	Gentamicin (CN)	10 µg	7 (7.8%)	22 (24.4%)	χ2=9.249	0.004*
	Tetracyclines	Doxycycline (DOX)	30 µg	29 (32.2%)	36 (40%)	χ2=1.180	0.352
Inhibition of cell DNA replication	Quinolones	Ciprofloxacin (CIP)	5 µg	14 (15.6%)	27 (30%)	χ2=5.338	0.032*
Inhibition of folic acid metabolism	Folate pathway antagonists	Trimethoprim-Sulfamethoxazole (SXT)	25/23.75 µg	28 (31.1%)	29 (32.2%)	Fissure Exact = 0.995	0.873

χ2= Pearson Chi-Square test. P value is considered significant if ≤0.05.
P value is considered highly significant if ≤0.005

Table (7): Multidrug resistance profile of *E. coli* isolated from the 180 COVID-19 and non-COVID-19 studied patients

Drug resistance	COVID-19 n=90		Non-COVID-19 n=90		Analytic test	P value
	N	%	N	%		
MDR	68	55.3%	55	44.7%	χ^2=4.339	0.037*
Not MDR	22	38.6%	35	61.4%		

χ2= Pearson Chi-Square test. P value is considered significant if ≤0.05.
P value is considered highly significant if ≤0.005

Discussion

The global community continues to grapple with the aftermath of the COVID-19 pandemic, which originated in Wuhan, Hubei, China, at a seafood market. From its initial detection in 2019 to October 2022, the pandemic tragically resulted in nearly 6 million deaths worldwide. Additionally, a staggering 755,703,002 confirmed cases of COVID-19 were reported during this period.[18]

In our study, the patients' age within the COVID-19 group varied between 18 and 79 years. The most affected age group was the group from 30 up to < 45 years (34.4%), followed by the age group from 18 to < 30 years (31.1%) as shown in table (4). These findings are in agreement with the Centers for Disease Control and Prevention (CDC) report in September 2020. This report stated that during June–August 2020, the COVID-19 prevalence was greater in young adults (20–29 years) accounting for more than 20% of all confirmed COVID-19 cases. [19]

Globally, numerous epidemiological reports have established that the change in age distribution occurred in a time-dependent pattern. As the pandemic first began, the majority of COVID-19 patients were adults. Then, the virus was simultaneously spreading to younger people as the COVID-19 variant was progressing. [20]

Ahmad S [21] have stated that the observed differences within the age profile between the different patient's groups were confounded by several factors as follows: the degree and target of tested population, ethnic variations from one place to the other, and age variations within the general population.

Several concerned reports of the COVID-19 infection have abandoned the possible sex-related influences although sex differences were described in both diagnosis and testing of COVID-19.[22]

In agreement with Sama et al. [23], our study findings showed that male patients were more affected by COVID-19 than females (58.9% and 41.1% respectively) as illustrated in tables (3 and 4). It has been observed that men, globally, have a relatively greater consumption rate of both smoking and alcohol. Moreover, men are more susceptible to high-risk behaviors and certain occupational hazards such as transportation, construction, manufacturing delivery and food processing than women. This is further elevating men's likelihoods to contract the COVID-19. [24]

On the other hand, several reports have confirmed that women display greater adherence to the COVID-19 linked controlling measures, such as face mask-wearing, social distancing, hand washing, and restrictions on movement. These COVID-19 proper behaviors aid in protecting individuals from COVID-19 contracting. [25]

In the present study, azithromycin was significantly administered to the COVID-19 patients (82.2%) compared to the non-COVID-19 patients (8.88%) with $P \leq 0.001$ as shown in table (5). It is worth mentioning that in COVID-19 infection, azithromycin is approved by the Egyptian Ministry of Health and Population (MOHP) to be among the treatment protocol modalities for the pandemic. [26]

In their work, Kamara et al. [27] have reported similar results where azithromycin was the most commonly supplemented antibiotic (34.8%). In contrast, Saleem et al. [28] reported that among COVID-19 patients, the top 3 most frequently prescribed antimicrobials were ceftriaxone (26.6%), metronidazole (9.7%) and vancomycin (7.9%). Their study was performed in Pakistani hospitals where ceftriaxone was usually used as intravenous injection.

Considering using azithromycin in COVID patients' treatment, Butler et al. [29] have stated that azithromycin must not be routinely utilized to manage COVID-19 in the community, in lack of further indications. Their findings have had vital stewardship implications for antibiotic throughout this pandemic. It indicated that the inadequate antibiotics usage could lead to augmented resistance for the antibiotic.

As a severe worldwide threat, AMR is regarded as growing health care concern. Unfortunately, this matter has not received sufficient attention throughout the last COVID-19 pandemic. During the COVID-19 pandemic, researchers have discovered a noteworthy decline in AMR surveillance. This might have restricted the capability to provide data on AMR definite alterations and it raised the likelihood of having a silent AMR pandemic. [30]

In this study, *E. coli* was isolated from stool samples for examining its antibiotic resistance profile. *E. coli* is one of the most globally investigated bacteria. Debatably, it is the best comprehended of the whole model microorganisms. [31]

The extrinsic (acquired) resistance along with constantly rising *E. coli* resistance to antibiotics is presently regarded as a major public health issue worldwide. One of the main factors contributing to the current antibacterial resistance dilemma is the fast evolution of bacteria under selective antibiotic pressure. Since a key factor contributing to the rise in MDR strains of bacteria is the ongoing interaction between any specific antibiotic and the bacterium. Regrettably, the inappropriate prescription and overuse of these medications are 2 large contributors to such problem. [10]

Our study showed (in table 6) that AMR of *E.coli* was detected in both COVID-19 and non-COVID-19 groups with no significant difference except for ertapenem which was observed in COVID group vs non-COVID group by (8.9% vs 0%, P = 0.007) and on the other hand, significant higher resistance was observed in non-COVID group considering gentamicin (24.4% vs 7.8%, P = 0.004), ciprofloxacin (30% vs 15.6%, P = 0.032) and aztreonam (36.7% vs 21.1%, P = 0.032).

These results are in agreement with Kishk et al. [32]. They have reported that on comparison between antibiotic susceptibility of gram-negative organisms within the non-COVID-19 and during the COVID-19 era, there was not any significant differences between the studied groups regarding ceftriaxone, cefepime, ampicillin and trimethoprim.

Even though quinolones are a well-known class of broad-spectrum antibiotics, the current study stated resistance rate was rather high (30%). There are growing concerns about the widespread overuse of these broad-spectrum antibiotics in developing nations, which could result in a notable rise in the rate of resistance. Bolon et al., for example, have documented a constant, stepwise rise in *E. coli* resistance to ciprofloxacin. [33]

In our study, carbapenems continued to be the most effective medication against *E. coli.* This is consistent with the findings of Garcia et al. [34], who reported that carbapenem resistance was not observed. Carbapenem resistance deteriorates with time, posing threats to the infected individuals and serving as some MDR isolates' sole antibiotic option. More than 50% of the reported isolates of *E. coli* to the European Centre for Disease Prevention and Control (ECDC) in 2018 demonstrated resistance to at least one antimicrobial group under monitoring, and MDR was common. [35]

In our research, the MDR *E. coli* isolates were considerably greater in the COVID-19 individuals (55.3%) in comparison to the non-COVID-19 individuals (44.7%) (P =0.037), though both findings were high (as shown in table 7). MDR is a reflection of the widespread use of antibiotics, and its great prevalence is due to the organism's capacity to acquire resistance genes. [36]

These results are in line with Bogossian et al. [37] who evaluated the acquisition of MDR bacteria in patients with COVID-19. Their MDR percentage was 54%, which is extremely close to the MDR percentage in our study's COVID-19 patients (55.3%).

These findings differed from those of Alali et al. [38], who found that the MDR prevalence of *E. coli* isolated from COVID-19 medical wards was 37.4%, with no noticeable difference from non-COVID-19 wards.

Overusing antibiotics is considered the main cause of antibiotic resistance, which might affect antibiotic stewardship and hence worsen the AMR.

Conclusion

E. coli isolated from stool samples of COVID-19 patients have shown a significantly higher MDR profile than non-COVID-19 patients. There was a substantial difference in azithromycin usage between COVID-19 and non-COVID-19 patients, with COVID-19 patients exhibiting higher usage rates. A detected high resistance to the broad-spectrum quinolones was observed in both COVID-19 and non-COVID-19 patients, while the most efficient drugs against *E. coli* were the Carbapenems in both groups.

Recommendations

Antibiotic use must be restricted without the clinician appropriate indication particularly those antibiotics used as a last resort. MDR organisms in the community necessitate the importance of adopting educational programs to raise awareness about the hazards of antibiotic abuse including bacterial resistance.

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