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Original article

Pattern of antimicrobial resistance in the pre and during COVID-19 era: An observational study

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ABSTRACT

Background: World Health Organization has documented the exaggerated use of broad-spectrum antibiotics during the COVID-19 pandemic raising warnings of increasing antimicrobial resistance. **Aim:** This is an observational cross-sectional comparative study that was done to describe the pattern of antibiotics resistance before and during the COVID-19 era to explain if this pattern is affected with using different antibiotics in COVID-19 era. **Methods:** Various clinical specimens from patients admitted in the urology, internal medicine, surgery inwards, intensive care unit and neonatal ICU in Suez Canal University Hospital in the pre-COVID-19 period (January 2019 to January 2020) and during COVID-19 pandemic (January 2020 to January 2021) were included. 627 patients,349 (55.6%) patients in the pre-COVID-19 era and 278 (44.4%) patients during the COVID-19 era. **Results:** Most samples were Gram-negative organisms (86%), while gram-positive represent 14% only. The most common Gram-negative isolates include *Escherichia coli (E. coli)* (30.9%). The most common Gram-positive is *Staphylococcus* species (11.8%). The study found a statistically significant increase in the resistance for cefazoline (p=0.002), nitrofurantoin (p=<0.001), aztreonam (p=<0.001) and tobramycin (p=<0.001) during the COVID-19 era compared with the pre-COVID-19 era. Among the Gram-negative pathogens, there is a significant increase in the resistance for ampicillin (p=0.023), ciprofloxacin (p=0.013), nitrofurantoin (p=<0.001), aztreonam (p=<0.001), tobramycin (p=0.035), trimethoprim -sulphamethoxazole (p=0.029), cefazoline (p=0.011), aztreonam (p<0.001), tigecyclin (p=0.048), and amikacin (p=0.043) during the COVID-19 compared with before, but the susceptibility pattern for the Gram-positive pathogens did not vary in both periods. **Conclusion:** COVID-19 pandemic led to the uncontrolled use of broad-spectrum antimicrobials, causing an increase in the antimicrobials resistance (AMR). Strict adherence to antimicrobial stewardship is essential.

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Introduction

Antimicrobials are the different classes of substances acting against different microorganisms [1]. Bacterial resistance to antibiotics is the bacterial cells' ability to overcome the antibiotic's bacteriostatic or bactericidal effects. The excessive abuse of antibiotics gives a chance of bacterial resistance to these antibiotics, which decreases the chance of curing diseases and achieving global medical coverage [2]

Antimicrobials resistance (AMR) may significantly deplete the host's immune system, increase antibiotic therapy ineffectiveness, and negatively affect the prognosis of the disease. AMR development is a multifactorial phenomenon that shares a common concept: selective antibiotics pressure on microbes [3]. Antibiotics attack susceptible microbes, so any bacteria that carry AMR genes can survive and persist despite the presence of antibiotics. The AMR genes spread among other bacteria "horizontal gene transfer", through the uptake of naked DNA material and mobile genetic elements such as plasmids, integrons, transposons, gene cassettes, and bacteriophages [4].

Although Systemic Acute Respiratory Syndrome Coronavirus (SARS-CoV) is a viral infection, it has been documented that 72% of infected cases admitting healthcare centres have received antibiotics, although 8% only are co-infected by bacteria or fungi [5]. Therefore, severe coinfections by strong drug-resistant and pan-resistant microbes have been reported in many cases [6]. The World Health Organization (WHO) has documented the exaggerated use of broad-spectrum antibiotics during Corona Virus Disease-19 (COVID-19), raising warnings of increasing AMR [7].

The COVID-19 pandemic is exacerbating AMR; data from five countries explained that 69% of COVID-19 diagnoses are associated with secondary bacterial infections, with higher prevalence in patients who are admitted to intensive care units (ICUs). However, a United States multicenter study reported that about 72% of COVID-19 patients received antibiotics even when not clinically indicated, which can promote AMR [8].

During COVID-19 pandemic, early reports showed high rates of multiple different antibiotic utilization in COVID-19 patients despite their lack of direct activity and efficacy against viral pathogens [9], and this unnecessary use of various antibiotics may lead to the development of antimicrobial resistance, a global public health crisis in the future.

In Egypt, the pattern of antibiotics resistance during the era of COVID-19 and after multiple uses of different antibiotics during different COVID-19 treatments protocols is not known and not studied, so in this study, we will describe the pattern of antibiotics resistance before and during the COVID-19 era to explain if this pattern is affected with using different antibiotics in COVID-19 era.

Patients and method

Type of the study and study population

This is an observational cross-sectional comparative study which was conducted in Ismailia Suez Canal University Hospital, Egypt. All patients admitted in the Urology, Internal Medicine, Surgery inwards, intensive care unit (ICU) and Neonatal ICU(NICU) in Suez Canal university hospital with different clinical conditions at the time of conducting the study before the COVID-19 pandemic spread (1 January 2019 to 1 January 2020) and during COVID-19 pandemic (2 January 2020 to January 2021) were included in the study.

Sampling

A comprehensive sample of all patients admitted in the Urology, Internal Medicine, Surgery inwards, intensive care unit (ICU) and Neonatal ICU (NICU) in Suez Canal university hospital at the time of conducting the study. The sample size was estimated by using the equation of two different proportions based on the pooled prevalence of AMR in the pre-pandemic (= 13 % [10] and during the COVID-19 pandemic era (=24%) [11]. A sample size of 210 patients for each group was estimated. After adding a non-response rate of 10%, 231 patients for each group were estimated.

Data collection tool

Sociodemographic data of the patients, the culture, and the sensitivity result of the various clinical specimens (Urine, sputum, blood, pus) were obtained from the patient's medical reports from the Clinical Pathology lab of Suez Canal University Hospital, due to the difficulty of getting the samples from the patients in the context of infection control measures in the COVID-19 period.

Specimens were collected correctly under aseptic conditions, and bacterial isolates were cultured on MacConkey and blood agar plates and incubated at 37°C for 18-24 hrs. Identification of the isolated microbe and their antibacterial susceptibility was done by using VITEC-2-Compact 15 for the following antibiotics: Ampicillin, ampicillin-sulbactam, benzylpenicillin, oxacillin, ceftriaxone, cefazoline, cefepime, imipenem, meropenem, ertapenem, amikacin, gentamicin, topramicin moxifloxacin, levofloxacin, ciprofloxacin, aztreonam, erythromycin vancomycin, tetracycline, tigecycline, clindamycin, linezolid, trimethoprim - sulphamethoxazole nitrofurantoin, rifampicin and quinupristin/dalfopristin

Operational definitions

MDR (multi-drug resistance): was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [12]. XDR (extensive drug resistance): was described as non-susceptibility to at least one agent in all but two or fewer antimicrobial types (i.e., bacterial isolates remain susceptible to only one or two categories) [12]. PDR (pan drug resistance): was defined as non-susceptibility to all agents in all antimicrobial categories [12].

Data management

Data was entered and analyzed using the statistical package for social science (SPSS) software version 23. The Kolmogorov-Smirnov test was used to assess the normality of the distribution of the studied variables. Quantitative data such as age was presented in mean and standard deviation. Qualitative data such as gender was presented as frequency and percentages. Comparison of AMR between pre and the COVID-19 groups for categorical variables was assessed using the Chi-square test (Fisher or Monte Carlo). Mann-Whitney test was used to compare two groups for not normally distributed quantitative variables. The significance of the obtained results was judged at the 5% level.

Ethical considerations

All procedures conducted in this study involving human materials were approved by the official ethical committee of the Suez Canal University before accomplishing the study. Written consent was obtained from each participant before specimen collection.

Results

This study included 627 patients,349 (55.6%) patients in the pre-COVID-19 era and 278 (44.4%) patients during the COVID-19 era. Our specimens were isolated from different surgical and non-surgical sites; the most common specimens were from urine (55.8%), sputum (24.7%), blood (6.7%) and pus 80 (12.8%). Most of our samples were Gram-negative organisms (86%), while Gram-positive represent 14% only of the total sample. The most common Gram-negative isolates include *E. Coli* (30.9%), *Klebsiella* (27.9%), *Acinetobacter* species (11%) and *Pseudomonas* species (8.3%). The most common Gram-positive is the *Staph* species (11.8%). From all these organisms only, *E. Coli* species growth significantly declined during the COVID-19 era, and *Burkholderia cepacia* growth significantly appeared during the COVID-19 period (p= 0.024 and 0.007), respectively **(Table 1).**

Comparison between the susceptibility of the different studied antimicrobial drugs in the pre and during the COVID-19 era are shown in **(Table 2 A&B).** Four antibiotics showed a statistically significant increase in resistance during the COVID-19 era compared with the pre-COVID-19 era, and cefazoline resistance increased from 79% in the pre-COVID-19 to 88.9% during the COVID-19 era (p=0.002). the resistance to nitrofurantoin increased from 30.7% in the pre-COVID-19 to 58.3% during the COVID-19 (p=<0.001).

The resistance to aztreonam increased from 37.4% pre-COVID-19 to 74.8% during COVID-19 (p=<0.001). The resistance to tobramycin increases from 40.7% pre-COVID-19 to 57.8% during COVID-19 (p=<0.001) **(Figure 1).** There is a statistically significant difference between the pattern of resistance in specimens collected pre-COVID-19 and during the COVID-19 era. XDR increased from 17.8% in the pre-COVID-19 era to 27% during the COVID-19 era (p=<0.001) **(Table 3).**

Among the Gram-negative pathogens, it was observed that there was a significant increase in the resistance to many drugs during the COVID-19 era than the pre-COVID-19 era for the following organisms: *Klebsiella pneumoniae* (ampicillin, ciprofloxacin, nitrofurantoin and aztreonam), followed by *E. Coli* (aztreonam, tobramycin and trimethoprim -sulphamethoxazole), *Pseudomonas* (cefazoline, aztreonam and tigecyclin), *Proteus* (ampicillin, amikacin and nitrofurantoin), but the susceptibility pattern for the remaining Gram-

negative organism did not vary in both periods **(Table 4),** while the susceptibility pattern for Gram-positive pathogens did not vary in both periods **(Table 5).**

Table 1. Sociodemographic characteristics of the studied participants in the pre and during the COVID-19 era.

	Total (n = 627)	**Pre COVID-19 (n = 349)**	**During the COVID-19 (n = 278)**	**Test of Sig.**	***P***
Gender					
Male	360 (57.4%)	193 (55.3%)	167 (60.1%)	χ^2= 1.441	0.230
Female	267 (42.6%)	156 (44.7%)	111 (39.9%)		
Age					
Mean ± SD.	57.51 ± 21.06	59.07 ± 20.13	55.56 ± 22.06	U= 45899.5	0.246
Median (Min. – Max.)	64.0 (0.1 – 95.0)	64.0 (0.1 – 95.0)	63.0 (0.1 – 90.0)		
Gram Stain					
Gram –ve	539 (86.0%)	305 (87.4%)	234 (84.2%)	χ^2= 1.330	0.249
Gram +ve	88 (14.0%)	44 (12.6%)	44 (15.8%)		
Specimen					
Urine	350 (55.8%)	202 (57.9%)	148 (53.2%)	χ^2= 65.460*	<0.001*
Sputum	155 (24.7%)	87 (24.9%)	68 (24.5%)		
Blood	42 (6.7%)	0 (0.0%)	42 (15.1%)		
Pus	80 (12.8%)	60 (17.2%)	20 (7.2%)		
Organism					
Gram –ve					
Klebsiella	175 (27.9%)	105 (30.1%)	70 (25.2%)	χ^2=1.851	0.174
E Coli	194 (30.9%)	121 (34.7%)	73 (26.3%)	χ^2=5.124*	0.024*
Acinetobacter species	69 (11.0%)	32 (9.2%)	37 (13.3%)	χ^2=2.708	0.100
Pseudomonas species	52 (8.3%)	28 (8.0%)	24 (8.6%)	χ^2=0.076	0.783
Morganella species	2 (0.3%)	0 (0.0%)	2 (0.7%)	χ^2= 2.519	^{FE}p=0.196
Chryseobacterium indologenes	1 (0.2%)	0 (0.0%)	1 (0.4%)	χ^2=1.257	^{FE}p=0.443
Proteus mirabilis	23 (3.7%)	12 (3.4%)	11 (4.0%)	χ^2=0.118	0.732
Providencia stuartii	1 (0.2%)	0 (0.0%)	1 (0.4%)	χ^2=1.257	^{FE}p=0.443
Burkholderia cepacia	6 (1.0%)	0 (0.0%)	6 (2.2%)	χ^2=7.605*	^{FE}p=0.007*
Citrobacter freundii	3 (0.5%)	1 (0.3%)	2 (0.7%)	χ^2= 0.609	^{FE}p=0.587
Serratia marcescen	1 (0.2%)	0 (0.0%)	1 (0.4%)	χ^2=1.257	^{FE}p=0.443
Enterobacter species	10 (1.6%)	4 (1.1%)	6 (2.2%)	χ^2=1.010	^{FE}p=0.351
Gram +ve					
Enterococcus species	11 (1.8%)	7 (2.0%)	4 (1.4%)	χ^2=0.289	^{FE}p=0.762
Streptooccus species	4 (0.6%)	2 (0.6%)	2 (0.7%)	χ^2=0.052	^{FE}p=1.000
Vagococcus fluvialis	1 (0.2%)	0 (0.0%)	1 (0.4%)	χ^2=1.257	^{FE}p=0.443
Staph species	74 (11.8%)	37 (10.6%)	37 (13.3%)	χ^2=1.090	0.297

SD: Standard deviation, U: Mann Whitney test , □2: Chi square test, FE: Fisher Exact p: p value for comparing between pre and during the COVID-19

*: Statistically significant at $p \leq 0.05$

Table 2A. Comparison between antibiotic susceptibility of Gram-negative organisms in the pre and during the COVID-19 era..

Antibiotics for gm-	Total	Pre COVID-19	During the COVID-19	χ^2	*P*
Ampicillin	**(n =539)**	**(n =305)**	**(n =234)**		
Sensitive	118 (20.9%)	61 (20.0%)	57 (21.9%)	0.314	0.575
	447 (79.1%)	244 (80.0%)	203 (78.1%)		
Ampicillin-sulbactam	**(n =539)**	**(n =305)**	**(n =234)**		
Sensitive	105 (19.5%)	53 (17.4%)	52 (22.2%)	1.982	0.159
	434 (80.5%)	252 (82.6%)	182 (77.8%)		
Ceftriaxone	**(n = 538)**	**(n = 3.4)**	**(n = 234)**		
Sensitive	104 (19.3%)	63 (20.7%)	41 (17.5%)	0.870	0.351
	434 (80.7%)	241 (79.3%)	193 (82.5%)		
Cefazoline	**(n = 539)**	**(n = 305)**	**(n = 234)**		
Sensitive	90 (16.7%)	64 (21.0%)	26 (11.1%)	9.278*	0.002*
	449 (83.3%)	241 (79.0%)	208 (88.9%)		
Cefipime	**(n = 538)**	**(n = 304)**	**(n = 234)**		
Sensitive	122 (22.7%)	61 (20.1%)	61 (26.1%)	2.717	0.099
	416 (77.3%)	243 (79.9%)	173 (73.9%)		
Imipenime	**(n = 538)**	**(n = 304)**	**(n = 234)**		
Sensitive	308 (57.2%)	179 (58.9%)	129 (55.1%)	0.761	0.383
	230 (42.8%)	125 (41.1%)	105 (44.9%)		
Meropenim	**(n = 539)**	**(n = 305)**	**(n = 234)**		
Sensitive	346 (64.2%)	205 (67.2%)	141 (60.3%)	2.788	0.095
	193 (35.8%)	100 (32.8%)	93 (39.7%)		
Ertapenim	**(n = 538)**	**(n = 305)**	**(n = 233)**		
Sensitive	322 (59.9%)	185 (60.7%)	137 (58.8%)	0.190	0.663
	216 (40.1%)	120 (39.3%)	96 (41.2%)		
Ciprofloxacin	**(n = 588)**	**(n = 313)**	**(n = 275)**		
Sensitive	212 (36.1%)	120 (38.3%)	92 (33.5%)	1.515	0.218
	376 (63.9%)	193 (61.7%)	183 (66.5%)		
Moxifloxacin	**(n = 626)**	**(n = 348)**	**(n = 278)**		
Sensitive	239 (38.2%)	143 (41.1%)	96 (34.5%)	2.817	0.093
	387 (61.8%)	205 (58.9%)	182 (65.5%)		
Nitrofurantoin	**(n = 564)**	**(n = 348)**	**(n = 216)**		
Sensitive	331 (58.7%)	241 (69.3%)	90 (41.7%)	41.832*	<0.001*
	233 (41.3%)	107 (30.7%)	126 (58.3%)		
Aztreonam	**(n = 536)**	**(n = 302)**	**(n = 234)**		
Sensitive	248 (46.3%)	189 (62.6%)	59 (25.2%)	74.057*	<0.001*
	288 (53.7%)	113 (37.4%)	175 (74.8%)		
Amikacin	**(n = 540)**	**(n = 306)**	**(n = 234)**		
Sensitive	354 (65.6%)	207 (67.6%)	147 (62.8%)	1.368	0.242
	186 (34.4%)	99 (32.4%)	87 (37.2%)		
Gentamicin	**(n = 627)**	**(n = 349)**	**(n = 278)**		
Sensitive	355 (56.6%)	201 (57.6%)	154 (55.4%)	0.304	0.581
	272 (43.4%)	148 (42.4%)	124 (44.6%)		
Topramicin	**(n = 263)**	**(n = 230)**	**(n = 493)**		
Sensitive	253 (51.3%)	156 (59.3%)	97 (42.2%)	14.431*	<0.001*
	240 (48.7%)	107 (40.7%)	133 (57.8%)		
Trimethoprim	**(n = 597)**	**(n = 347)**	**(n = 250)**		
Sensitive	211 (35.3%)	113 (32.6%)	98 (39.2%)	2.799	0.094
	386 (64.7%)	234 (67.4%)	152 (60.8%)		
Tigacycline	**(n = 624)**	**(n = 347)**	**(n = 277)**		
Sensitive	474 (76.0%)	268 (77.2%)	206 (74.4%)	0.693	0.405
	150 (24.0%)	79 (22.8%)	71 (25.6%)		

χ^2: Chi square test , MC: Monte Carlo, p: p value for comparing between pre and during the COVID-19

*: Statistically significant at $p \leq 0.05$

Table 2B. Comparison between antibiotic susceptibility of Gram-negative organisms in the pre and during the COVID-19 era.

Antibiotics for gm+	Total (n = 627)	Pre COVID-19 (n = 349)	During the COVID-19 (n = 278)	χ^2	p
Benzylpenicilin	**(n = 88)**	**(n = 44)**	**(n = 44)**		
Sensitive	19 (21.6%)	11 (25.0%)	8 (18.2%)	0.604	0.437
	69 (78.4%)	33 (75.0%)	36 (81.8%)		
Tetracycline	**(n = 88)**	**(n = 44)**	**(n = 44)**		
Sensitive	42 (47.7%)	19 (43.2%)	23 (52.3%)	0.729	0.393
	46 (52.3%)	25 (56.8%)	21 (47.7%)		
Oxacillin	**(n = 88)**	**(n = 44)**	**(n = 44)**		
Sensitive	31 (35.2%)	17 (38.6%)	14 (31.8%)	0.448	0.503
	57 (64.8%)	27 (61.4%)	30 (68.2%)		
Rifampicin	**(n = 88)**	**(n = 44)**	**(n = 44)**		
Sensitive	73 (83.0%)	37 (84.1%)	36 (81.8%)	0.080	0.777
	15 (17.0%)	7 (15.9%)	8 (18.2%)		
Linezolid	**(n = 88)**	**(n = 44)**	**(n = 44)**		
Sensitive	83 (94.3%)	41 (93.2%)	42 (95.5%)	0.212	0.645
	5 (5.7%)	3 (6.8%)	2 (4.5%)		
Erythromycin	**(n = 88)**	**(n = 44)**	**(n = 44)**		
Sensitive	38 (43.2%)	20 (45.5%)	18 (40.9%)	0.185	0.667
	50 (56.8%)	24 (54.5%)	26 (59.1%)		
Vancomicin	**(n = 88)**	**(n = 44)**	**(n = 44)**		
Sensitive	83 (94.3%)	43 (97.7%)	40 (90.9%)	1.908	^{FE}p= 0.360
	5 (5.7%)	1 (2.3%)	4 (9.1%)		
Quinupristin/dalfopristin	**(n = 88)**	**(n = 44)**	**(n = 44)**		
Sensitive	72 (81.8%)	36 (81.8%)	36 (81.8%)	0.00	1.000
	16 (18.2%)	8 (18.2%)	8 (18.2%)		
Clindamycin	**(n = 88)**	**(n = 44)**	**(n = 44)**		
Sensitive	56 (63.6%)	29 (65.9%)	27 (61.4%)	0.196	0.658
	32 (36.4%)	15 (34.1%)	17 (38.6%)		
Levofloxacin	**(n = 88)**	**(n = 44)**	**(n = 44)**		
Sensitive	61 (69.3%)	28 (63.6%)	33 (75.0%)	1.336	0.248
	27 (30.7%)	16 (36.4%)	11 (25.0%)		

χ^2: Chi square test, MC: Monte Carlo , p: p value for comparing between pre and during the COVID-19

Table 3. Comparison between pre and during the COVID-19 according to the type of resistance

Type of resistance	Total (n = 627)	Pre COVID-19 (n = 349)	During the COVID-19 (n = 278)	χ^2	^{MC}p
MDR	254 (40.5%)	166 (47.6%)	88 (31.7%)	29.871*	<0.001*
XDR	137 (21.9%)	62 (17.8%)	75 (27.0%)		
PDR	49 (7.8%)	36 (10.3%)	13 (4.7%)		
None	187 (29.8%)	85 (24.4%)	102 (36.7%)		

χ^2: Chi square test, MC: Monte Carlo, p: p value for comparing between pre and during the COVID-19

Table 4. Comparison of antimicrobial susceptibility pattern of Gram-negative bacteria between pre-COVID-19 and COVID-19 period.

Antibiotics	Organism (Gram -ve)																			
	Klebsiella		E Coli		Acinetobacter species		Pseudomonas species		Proteus mirabilis		Burkholderia cepacian		Citrobacter freundii		Serratia marcescen		Enterobacter species	
	Pre (n=105)	During the (n=70)	Pre (n=121)	During the (n=73)	Pre (n=32)	During the (n=37)	Pre (n=28)	During the (n=24)	Pre (n=12)	During the (n=11)	Pre (n=0)	During the (n=6)	Pre (n=1)	During the (n=2)	Pre (n=0)	During the (n=1)	Pre (n=4)	During the (n=5)
Ampicillin	84.8	95.7	83.5	87.7	84.4	67.6	60.7	75.0	33.3%	81.8%	-	66.7%	100	0	-	0	50.0	66.7
P	**0.023***		**0.427**		**0.106**		**0.274**		**0.036***		-		**0.333**		-		**1.00**	
Ampicillin-sulbactam	83.8%	81.4%	79.3%	69.9%	81.3%	70.3%	60.7%	75.0%	58.3%	54.5%	-	66.7%	100	0	-	0	75.0	80.0
P	**0.682**		**0.136**		**0.291**		**0.274**		**1.000**		-		**0.333**		-		**1.00**	
Ceftriaxone	76.2%	81.4%	78.5%	84.9%	87.1%	70.3%	57.1%	75.0%	33.3%	63.6%	-	66.7	100	0	-	-	50.0%	80.0%
P	**0.410**		**0.270**		**0.096**		**0.177**		**0.146**		-		**0.333**		-		**0.524**	
Cefazoline	80.0%	82.9%	77.7%	84.9%	96.9%	100.0%	75.0%	100.0%	33.3%	72.7%	-	100.0	100	100	-	100.0	75.0%	100.0%
P	**0.636**		**0.218**		**0.464**		**0.011***		**0.059**		-		-		-		**0.444**	
Cefipime	79.0%	78.6%	73.6%	79.5%	87.5%	73.0%	75.0%	54.2%	33.3%	63.6%	-	66.7	100	0	-	-	75.0%	60.0%
P	**0.940**		**0.353**		**0.135**		**0.115**		**0.146**		-		**0.333**		-		**1.000**	
Meropenim	43.8%	51.4%	7.4%	13.7%	71.9%	62.2%	42.9%	62.5%	8.3%	18.2%	-	-	-	-	-	-	25.0%	40.0%
P	**0.322**		**0.155**		**0.393**		**0.158**		**0.590**		-		-		-		**1.000**	
Imipenime	46.2%	50.0%	17.4%	13.7%	71.9%	62.2%	53.6%	66.7%	25.0%	63.6%	-	66.7	-	-	-	-	50.0%	40.0%
P	**0.618**		**0.501**		**0.393**		**0.337**		**0.100**		-		-		-		**1.000**	
Ertapenim	51.4%	52.9%	12.4%	12.3%	87.5%	63.9%	46.4%	66.7%	8.3%	0.0%	-	66.7	-	-	-	-	25.0%	40.0%
P	**0.853**		**0.989**		**0.025***		**0.143**		**1.000**		-		-		-		**1.000**	
Ciprofloxacin	54.3%	72.9%	60.8%	68.5%	78.1%	73.0%	53.6%	75.0%	25.0%	54.5%	-	66.7	-	-	-	-	50.0%	33.3%
P	**0.013***		**0.283**		**0.620**		**0.110**		**0.214**		-		-		-		**1.000**	
Moxifloxacin	62.9%	74.3%	62.5%	67.1%	75.0%	70.3%	50.0%	75.0%	33.3%	54.5%	-	66.7	0.0	50.0	-	100.0	50.0%	33.3%
P	**0.114**		**0.516**		**0.661**		**0.065**		**0.414**		-		**1.000**		-		**1.000**	
Nitrofurantoin	40.4%	69.8%	11.6%	21.9%	65.6%	62.5%	46.4%	70.0%	41.7%	90.9%	-	66.7	0.0	50.0	-	-	25.0%	33.3%
P	**<0.001***		**0.063**		**0.794**		**0.105**		**0.027***		-		**1.000**		-		**1.000**	
Aztreonam	42.9%	80.0%	23.1%	80.8%	68.8%	67.6%	46.4%	75.0%	16.7%	45.5%	-	83.3	-	-	-	-	25.0%	80.0%
P	**<0.001***		**<0.001***		**0.916**		**0.036***		**0.193**		-		-		-		**0.206**	
Amikacin	39.0%	35.7%	3.3%	2.7%	71.9%	64.9%	39.3%	58.3%	8.3%	45.5%	-	66.7	-	-	-	-	0.0%	40.0%
P	**0.656**		**1.000**		**0.533**		**0.171**		**0.043***		-		-		-		**0.444**	
Gentamicin	44.8%	40.0%	27.3%	31.5%	62.5%	59.5%	53.6%	62.5%	16.7%	18.2%	-	66.7	100.0	0.0	-	-	50.0%	50.0%
P	**0.533**		**0.528**		**0.796**		**0.516**		**1.000**		-		**0.333**		-		**1.000**	
Topramicin	45.7%	58.6%	20.6%	35.2%	63.3%	61.1%	56.0%	66.7%	10.0%	.0%	-	66.7	-	-	-	-	25.0%	40.0%
P	**0.104**		**0.035***		**0.853**		**0.444**		**1.000**		-		-		-		**1.000**	
Trimethoprim	73.3%	72.9%	72.7%	57.5%	68.8%	59.5%	59.3%	75.0%	41.7%	72.7%	-	16.7	-	-	-	-	50.0%	20.0%
P	**0.944**		**0.029***		**0.423**		**0.234**		**0.214**		-		-		-		**0.524**	
Tigacycline	18.1%	17.1%	1.7%	1.4%	21.9%	8.1%	57.7%	83.3%	33.3%	80.0%	-	33.3	-	-	-	-	0.0%	16.7%
P	**0.872**		**1.000**		**0.170**		**0.048***		**0.043***		-		-		-		**1.000**	

Table 5. Comparison of antimicrobial susceptibility pattern of Gram-positive bacteria between pre-COVID-19 and COVID-19 period.

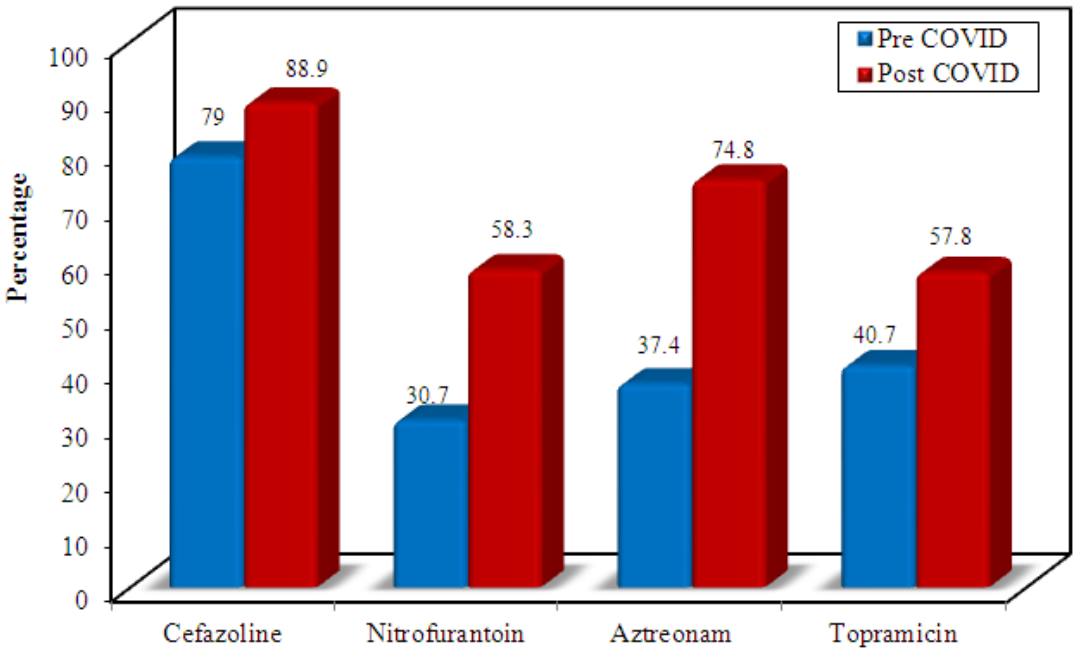
Antibiotics	**Organism (Gram +ve)**					
	***Enterococcus* species**		***Streptooccus* species**		***Staph* species**	
	Pre COVID-19	**During the COVID-19**	**Pre COVID-19**	**During the COVID-19**	**Pre COVID-19**	**During the COVID-19**
Ampicillin	-	25.0	-	50.0	100.0	31.6
P	**-**		**-**		**0.133**	
Ampicillin-sulbactam	0	25.0	-	-	100.0	100.0
P	**1.000**		-		-	
Ciprofloxacin	-	-	-	-	50.0	26.5
P	-		-		**0.247**	
Moxifloxacin	42.9	50.0	0	0	29.7	24.3
P	**1.000**		-		**0.601**	
Nitrofurantoin	-	-	0	0	5.4	20.0
P	-		-		**0.323**	
Aztreonam	-	-			66.7	100.0
P	-				**1.000**	
Amikacin	-	-			66.7	0.0
P	-				**1.000**	
Gentamicin	42.9	25.0	-	-	35.1	32.4
P	**1.000**		-		**0.806**	
Trimethoprim	42.9	0.0	-	50.0	44.4	31.3
P	**1.000**		-		**0.371**	
Benzylpenicilin	14.3	25.0	0	50.0	91.4	88.9
P	**1.000**		**1.000**		**1.000**	
Tetracycline	100.0	100.0	50.0	100.0	48.6	38.9
P	-		**1.000**		**0.411**	
Oxacillin	0.0	50.0	0.0	50.0	77.1	69.4
P	**0.109**		1.000		**0.464**	
Rifampicin	0.0	50.0	-	-	14.3	11.1
P	**0.109**		-		**0.735**	
Linezolid	14.3	25.0	0.0	50.0	0.0	0.0
P	**1.000**		**1.000**		-	
Erythromycin	71.4	75.0	0.0	50.0	45.7	55.6
P	**1.000**		**1.000**		**0.407**	
Vancomicin	0.0	25.0	0.0	50.0	2.9	0.0
P	**0.364**		**1.000**		**0.493**	

Table (5): "continue"

Antibiotics	Organism (Gram +ve)							
	***Enterococcus* species**		***Streptooccus* species**		***Vagococcus fluvialis***		***Staph* species**	
	Pre COVID-19	**During the COVID-19**	**Pre COVID-19**	**During the COVID-19**	**Pre COVID-19**	**During the COVID-19**	**Pre COVID-19**	**During the COVID-19**
Quinupristin/dalfopristin	100.0	100.0	0.0	50.0	-	-	2.9	0.0
P	-		**1.000**		-		**0.493**	
Clindamycin	28.6	50.0	50.0	50.0	-	100.0	31.4	30.6
P	**0.576**		**1.000**		-		**0.937**	
Levofloxacin	57.1	50.0	0	0	-	-	22.9	22.2
P	**1.000**		-		-		**0.949**	
Benzylpenicilin	14.3	25.0	0	50.0	-	100.0	91.4	88.9
P	**1.000**		**1.000**		-		**1.000**	
Tetracycline	100.0	100.0	50.0	100.0	-	-	48.6	38.9
P	-		**1.000**		-		**0.477**	
Oxacillin	0.0	50.0	0.0	50.0	-	100.0	77.1	69.4
P	**0.109**		**1.000**		-		**0.464**	
Rifampicin	0.0	50.0	-	-	-	100.0	14.3	11.1
P	**0.109**		-		-		**0.735**	
Linezolid	14.3	25.0	0	50.0	-	-	-	-
P	1.000		**1.000**		-		-	
Erythromycin	71.4	75.0	0.0	50.0	-	-	45.7	55.6
P	**1.000**		**1.000**		-		**0.407**	
Vancomicin	0.0	25.0	0	50.0	-	-	2.9	0.0
P	**0.364**		**1.000**		-		**0.493**	
Quinupristin/dalfopristin	100.0	100.0	0	50.0	-	-	2.9	0.0
P	-		**1.000**		-		**0.493**	
Clindamycin	28.6	50.0	50.0	50.0	-	100.0	31.4	30.6
P	**0.576**		**1.000**		-		**1.000**	
Levofloxacin	57.1	50.0	-	-	-	-	22.9	22.2
P	**1.000**		-		-		**1.000**	

χ^2: : Chi square test MC: MonteCarlo

Figure 1. Frequency distribution of antibiotics showed a statistically significant increase in the resistance during the COVID-19 era compared with pre-COVID-19 era.



Discussion

The COVID-19 pandemic has caused severe economic problems and a significant challenge in healthcare settings. The COVID-19 pandemic led to the uncontrolled use of broad-spectrum antimicrobials with multiple drug combination regimens to patients admitted to wards and in ICUs, due to the fear of getting COVID-19 infection and hesitancy to enter COVID-19 ICU wards. Over-the-counter availability of drugs, their improper use, the inability to differentiate between viral and bacterial associated respiratory complications, a delay time of culture reports, and the severity of manifestation among patients prompted clinicians to start a presumptive antibiotic, which eventually led to antimicrobial resistance. In Egypt, one of the developing countries, little published information on antimicrobial susceptibility and change in resistance patterns during the COVID-19 pandemic is known.

In this study, 86.4% of isolated organisms were Gram-negative. *Escherichia coli (E. coli)* represented the majority of Gram-negative isolates (30.9%) followed by *Klebsiella* (27.9%), but the rate of infection with *E. coli* significantly decreased during COVID-19 pandemic (p=0.024), and this might be related to empirical use of antibiotics during respiratory infection in the COVID-19 pandemic which were active against *E. coli* as ceftriaxone and quinolones. *Burkholderia cepacia* species appeared to be isolated during COVID-19 pandemic (p=0.007). This can be explained by the update and improvement of isolation skills and isolation equipment, which started to be used during COVID-19 pandemic.

On the other hand, Gram-positive bacteria represent only 14.4% of all isolates, *Staphylococcus aureus* were predominant, followed by *Enterococci* (11.8%, and 1.8%, respectively). It was noted that there was no change in the rate of growth of gram-positive bacteria during COVID-19 pandemic despite the wide use of antimicrobials in the management of COVID-19.

In our study, among the beta-lactam drugs, cefazoline and aztreonam showed a significant increase in resistance from 79%, 37.4% in the pre-COVID-19 era to 88.9%, 74.8% during the COVID-19 era, respectively. Penicillin groups were widely used in Egypt in treatments of variable Gram-positive and some Gram-negative organisms and mostly used empirically without susceptibility base, So the rate of total resistance to agents in this group was high in our study (benzylpenicillin, ampicillin, ampicillin-sulbactam, oxacillin, 78.4%,78.9%,76.3%, 64.8% respectively), consequently, the pattern of resistance of these drugs not significantly changed during COVID-19

pandemic. The current study found that from the carbapenems beta-lactam antimicrobials (imipenem, meropenem, ertapenem), which are considered as a broad-spectrum agent, meropenem showed a marginally significant increase in the rate of resistance during COVID-19 pandemic (p=0.053) as its widely used in suspected or confirmed COVID-19 patients during COVID-19 pandemic.

A retrospective study was done in NICU, pediatric ICU (PICU) and adult ICU in México which reported that Gram-negative bacteria exhibited a high resistance rate for ampicillin (95.85%), cefuroxime (84.17%), piperacillin (82.93%), cefotaxime (78.07%), ceftriaxone (77.41%), aztreonam (75.23%), cefazolin (75.00%), and ceftazidime (73.19%)[13] .

A recent Egyptian study detected ESBL-encoding genes in 75.4% of bacterial isolates indicating high resistance to cephalosporins in Egypt, which matched our results[14]. Meanwhile, a high level of carbapenem-resistant Gram-negative bacteria with 50.8% of the isolates harbouring at least one carbapenem resistance gene was reported in a recent Egyptian study [15], and these results were matched with our results.

In Brazil, Gaspar and his colleagues reported that the rate of *Acinetobacter baumannii* and *Klebsiella pneumoniae* resistance to carbapenems significantly increased in 2020 compared to pre COVID-19 pandemic, with a rate (78.6% and 62.1 %) respectively during the COVID-19 are [16]. Although another study performed in 2021 showed that the resistance rate of most gram-negative to carbapenem slightly increased during the COVID-19 pandemic but was also not statistically significant, these results match our results [17]. The previous study showed that among the aminoglycosides, the Tobramycin resistance pattern significantly increased from 40.7% pre-COVID-19 to 57.8% during the COVID-19 pandemic (p=0.017). In contrast, the resistance pattern to both amikacin and gentamicin showed no significant changes in resistant pattern. This finding may be explained as the use of aminoglycosides is a narrow spectrum and only used for aerobic Gram-negative bacilli, and their clinical utility is limited due to their serious toxicities as renal and ototoxicity and also not widely used except in urinary tract infections and not included in COVID-19 treatment protocol [17].

A similar study conducted by **Saini et al.** showed that the sensitivity of Gram-negative bacteria isolated from blood samples as *E. Coli* and *Pseudomonas aeruginosa* have a good susceptibility to aminoglycosides as gentamicin and amikacin pre COVID-19 and also no significant variation in susceptibility during the COVID-19 era, except sensitivity of *E. coli* and *Pseudomonas aeruginosa* to amikacin increased from 76 % and 66% pre-COVID-19 to 100% during the COVID-19 era respectively and the pattern of resistance to gentamicin is matched with our results, also his results showed that all Gram-negative isolates from urine samples (*Escherichia Coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Acinetobacter baumannii*) have a good susceptibility to aminoglycosides (both gentamicin and amikacin) and the rate and pattern of resistance not significantly altered during COVID-19 pandemic and this results matched with our results [17].

Our study included second, third and fourth quinolones (ciprofloxacin, levofloxacin, and moxifloxacin), respectively. The general resistance rate is high for ciprofloxacin and moxifloxacin (59.5%, 59.6%) and low for levofloxacin (25.0%), respectively, with no significant alteration in the resistance pattern during COVID-19 pandemic. The wide use of fluoroquinolones in Egypt during the COVID-19 pandemic and pre pandemic period in infections such as H. pylori, typhoid fever, and chest and urinary tract infections might explain this high rate of fluoroquinolone resistance in both periods. A study conducted by **Saini et al.** showed the susceptibility of Gram-positive and Gram-negative isolates to ciprofloxacin was low, and the resistance pattern did not significantly change during the COVID-19 periods for most isolates except for Klebsiella pneumoniae [17].

In this study, the general sensitivity of isolates to nitrofurantoin (antibiotic most specifically used in uncomplicated urinary tract infection) is good (62.6%), and unfortunately and surprisingly, the pattern of resistance significantly increased during the COVID-19 pandemic (p=<0.001), this may be explained that most of our specimens are urine samples. A study conducted by Saini et al. showed that the Gram-positive and Gram-negative isolates from urine samples had a high susceptibility rate to nitrofurantoin in both pre and during the COVID-19 pandemic except for *Klebsiella pnemoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* that had a high resistance rate and the rate of resistance not

significantly changed during COVID-19 pandemic [17].

Linezolid still has a good general sensitivity rate (96.6%) despite its wide use during the COVID-19 pandemic, as in the pre-COVID-19 area the use of linezolid was exclusively used in resistant infections of Gram-positive bacteria as a chest infection, methicillin resistance *Staphylococcus aureus* (MRSA) and despite its wide use during COVID-19 pandemic resistance pattern not changed during COVID-19 pandemic. A study conducted by Saini et al. showed that the general susceptibility of gram-positive isolates was high to linezolid either pre or during the COVID-19 pandemic, and these results matched our study [17]. However, **Sinai et al.** showed that isolates' resistance patterns significantly increased during the COVID-19 pandemic. These results are logical due to massive and marked abuse of linezolid during the COVID-19 pandemic, and the results of our study may have a small sample size, which is not representative of all the categories of patients.

Furthermore, we observed a predominance of Gram-negative organisms (86%). Among the Gram-negative pathogens, there is a significant increase in the resistance to many drugs during the COVID-19 era than the pre-COVID-19 era in the following organisms: *Klebsiella pneumoniae* for (ampicillin, ciprofloxacin, nitrofurantoin and aztreonam), then *E. coli* for (aztreonam, tobramycin and trimethoprim-sulphamethoxazole), *Pseudomonas* for (cefazoline, aztreonam and tigecyclin), *Proteus* for (ampicillin, amikacin and nitrofurantoin), but the susceptibility pattern for the remaining Gram-negative organism did not vary in both periods, while the susceptibility pattern for Gram-positive pathogens did not vary in both periods. The predominance of Gram-negative pathogens may be due to the fact that most of our specimens are urine samples.

In a study conducted in tertiary care hospital in Delhi, India, among the Gram-negative bacteria, *Acinetobacter baumannii* was the predominant bacteria isolated during COVID-19 compared to the pre–COVID-19 period with reduced susceptibility to gentamicin, amikacin and ciprofloxacin but an alarming decline in susceptibility was observed for Cotrimoxazole and piperacillin-tazobactam [17]. While in 2019 **Uc-Cachón et al.** reported that the enterobacterial isolates of *K. pneumoniae*, *E. coli* revealed high resistance rates to penicillins (97.12–75.00%), cephalosporins (100–59.37%) except for cefotetan, aztreonam (88.89–87.50%), and tobramycin (79.01–48.39%). Additionally, clinical isolates of *E. coli* displayed high resistance rates to fluoroquinolones (78.31–70.00%) [13].

The clinical isolates of *Acinetobacter baumannii* exhibited high resistance rates to third- and fourth generation cephalosporins (81.13–67.92%), ciprofloxacin (79.25%), gentamicin (84.91%), and trimethoprim/sulfamethoxazole (77.36%). The clinical isolates of *Pseudomonas aeruginosa* revealed high resistance rates to piperacillin (70.59%) and imipenem (69.68%). Other Gram-negative bacteria that included *Proteus mirabilis*, *Serratia marcescens*, *Burkholderia* spp., *Acinetobacter lwoffi*, and *Klebsiella oxytoca* revealed high resistance rates to ampicillin (100%), cefazolin (80.00%), cefuroxime (81.82%), ceftazidime (66.67%), and aztreonam (66.67%) [13].

Conclusion

Our study showed that, among the Gram-negative organisms, It was observed that there was a significant increase in the resistance to many antimicrobial drugs during the COVID-19 era compared with the pre-COVID-19 era for (Ampicillin, Ciprofloxacin, Nitrofurantoin, Aztreonam, Tobramycin, Trimethoprim - Sulphamethoxazole, Cefazoline, Aztreonam, Tigecycline and Amikacin), but the susceptibility pattern for the Gram Positive organisms did not vary in both periods. The COVID-19 pandemic led to the uncontrolled use of broad-spectrum antimicrobials with multiple drug combination regimens by the patients due to the fear of getting COVID-19 infection and hesitancy to enter COVID-19 ICU and wards. It is feared that the unrationalized use of antimicrobials could cause the next global public health crisis caused by antimicrobial resistance. So, to successfully combat AMR, it is important to emphasize the rational prescribing of antibiotics as a part of an antimicrobial stewardship program.

Limitations of the study

The main limitations of the present study were that sociodemographic data of the patients, the culture and the sensitivity result of the specimens had been obtained from the patient's medical reports due to the difficulty of obtaining the samples from the patients in the context of infection control measures in the COVID-19 period. Some clinical data of

patients, including antibiotics administered to the patients, results of treatment and mortality rate, are deficient. The classification of infections into (hospital or community-acquired infections) was not assessed.

Recommendations

Health policy makers should implement policies against inappropriate use or self-medication with antimicrobials for COVID-19-related symptoms. Knowledge about antimicrobial stewardship should be raised especially in resource-limited settings. The healthcare student's curriculum should include antimicrobial stewardship courses to improve antimicrobial use in future and prevent the emergence of AMR. Further studies about AMR should be implemented on a large scale to provide baseline data about the current situation of AMR.

Conflict of interest

No conflict of interest was declared.

Financial disclosure

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