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Original Article

Drug-Resistant Trends of *Acinetobacter Spp* Before and During the COVID-19 Pandemic in Punjab, Pakistan

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ABSTRACT

The escalating level of antimicrobial resistance in Pakistan poses a significant threat to public health nationwide. **Objective:** To evaluate the antibiotic resistance trend of *Acinetobacter spp* before and during the COVID-19 pandemic and differences in antimicrobial resistance rates. **Methods:** This study assessed the microbiological data in two periods: before COVID-19 (January 2017- March 2020) and during the COVID-19 period (April 1, 2020- March 31, 2021). Antibiotic sensitivity testing was performed by using the Kirby-Bauer disc diffusion technique. **Results:** Out of 625 strains of *Acinetobacter,* 462 (73.9%) were isolated in the pre-COVID-19 period and 163 (26.0%) during the COVID-19 period. The percentages of females in the pre COVID-19 and during COVID-19 era were 53%, and the proportion of males was 46% and 45%, respectively. The age group of 16-30 years (34%) was most infected in both periods. In the pre-COVID era, the percentage of *Acinetobacter spp* isolated from pus and urine was 47% and 34%, respectively, while in the COVID-19 period, it changed to 48% and 16%, respectively. The drugs that showed a significant increase in resistance during the COVID-19 period were Imipenem 53%, Aztreonam 91% to 100%, Ciprofloxacin 65% to 75%, Moxifloxacin 66% to 100%, Cefotaxime 61% to 97% and Tazobactam 61 to 71%. In the Lahore division, the infection rate increased from 35% (in the pre-COVID era) to 41% (during the COVID era). **Conclusions:** The notable differences in resistance patterns before and after the COVID-19 era indicate a decrease in the choices of drugs for *Acinetobacter* infections.

INTRODUCTION

Acinetobacter is gram-negative cocobacilli, a non-motile and aerobic bacterium that causes community and hospital-acquired infection [1]. *Acinetobacter spp* have appeared as severe multidrug resistant pathogens documented along with *Enterobacter species*, *Staphylococcus aureus, Klebsiella pneumoniae,* and *Enterococcus faecium,* collectively abbreviated as ESKAPE organisms, respectively, also named superbugs [2, 3]. Immunocompromised patients are more prone to *Acinetobacter* infection due to more exposure to broad spectrum drugs. Antibiotics of the different groups have different substances that act against pathogenic bacteria [4, 5]. The bacteriostatic or bactericidal effects of antibiotics against bacterial cells are called resistance or susceptibility. Previous outcomes from different places in the world described *Acinetobacter's* Inherent resistance to antimicrobial drugs through various ways like impermeability of the outer membrane, enzymatic inactivation, efflux pump systems, and biofilm formation, which leads to resistance to most of the groups like aminoglycosides, quinolones, and β-lactams [6, 7]. *Acinetobacter* strains are considered MDR (Multidrug-Resistant) if they show resistance to three or more different groups of drugs, XDR (extensively drug-resistant) strains are classified as if they are resistant to entirely available drugs excluding one or two, and PDR (Pan-Drug-Resistant) resistant to all if they are resistant to all existing antimicrobials Quraini MA *et al.*, in 2023 [8]. Infections with

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MDR *Acinetobacter* are considered a primary danger to universal health [9]. The extreme misuse of antibiotics contributes to bacterial resistance to most antibiotics, which declines the chance of curing infections [10]. Infection with multidrug resistance is expected to cause almost 10 million deaths and $100 billion in economic loss by Alkhodari SA and Elmanama AA in 2021 [11]. It is extensively recognized that antimicrobial analysis is critical for tackling antimicrobial resistance internationally [12]. In developing countries like Pakistan, public pharmacists deliver antibiotic drugs to the community without a physician's recommendation [13]. That's how it becomes a significant risk of spreading infectious diseases and, as a result, the severity of illness among immune-compromised people. Moreover, the costs of handling these infections have grown considerably [14, 15]. Thus, antimicrobial resistance is a significant cause of death internationally, with the maximum problems in low resource areas [16]. The COVID-19 pandemic also played a substantial role in accelerating the levels of antimicrobial resistance. Initial reports indicated that high amounts of various antibiotics were utilized during the COVID-19 pandemic, even though their efficacy against viral infections was very low, leading to a severe global health crisis [5, 17]. World Health Organization (WHO) declared the coronavirus disease (COVID-19) a health emergency on January 30, 2020, and announced an international pandemic on March 11, 2020 [18]. Most symptoms were dry cough, fever, and fatigue. During the COVID-19 pandemic, several antibiotics were promoted for treatment, like levofloxacin, amoxicillin-clavulanate, and azithromycin [19]. However, unnecessary antibiotic use in COVID-19 patients increased AMR [20]. The COVID-19 pandemic has had a significant and different influence on AMR in each country, according to their healthcare structure and community health strategy [21, 22]. Many factors such as studies on different populations, medical situations, and antibiotics recommending patterns, subsequently create a need to improve policies to overcome AMR [23].

This retrospective study has mainly aimed to evaluate the antibiotic resistance pattern of *Acinetobacter spp* isolated from different human samples before and during the COVID-19 pandemic, possible differences in antimicrobial resistance rates were assessed, multi-year drifts were created, and the antibiotics agent was considered to treat the *Acinetobacter* infection effectively.

METHODS

This retrospective study was conducted in a diagnostic laboratory collaborated with different tertiary care hospitals in Lahore, Pakistan, which determined the antibiotic resistance of *Acinetobacter spp* in pre and during COVID-19 pandemic eras. The designated study times were from January 2017 to March 2020 (pre-COVID-19) and April 2020 to March 2021 (during COVID-19). This study was conducted with the ethical approval of CitiLab & Research Centre (CRC 24-2/2021). Before sample collection, written consent was also obtained from each patient. The study comprised all the positive cultures for *Acinetobacter,* which were collected and recorded in microsoft excel during the pre-COVID-19 and COVID-19 periods. Sociodemographic data of the patients, like patient name, gender, age, area, clinical specimen types (like urine, sputum, blood, and pus), and susceptibility profiles of *Acinetobacter* were noted. The inclusion criterion of this study was only pure isolation of *Acinetobacter*. An exclusion criterion in this study was multi-microbial growth, incomplete data, gram-positive cocci, and other gram-negative bacteria except Acinetobacter. Under aseptic conditions, clinical specimens were collected and inoculated on culture media like MacConkey's agar, blood agar, chocolate agar, and CLED agar plates and incubated at 37°C overnight [23]. The Acinetobacter was identified by gram staining, colony morphology, and biochemical testing. Antibiotic Sensitivity Testing (AST) was performed by using the Kirby-Bauer disc diffusion technique on MHA following the CLSI guidelines [24]. Antimicrobial drugs tested for Acinetobacter involved; Ampicillin (AMP), Imipenem (IMP), Meropenem (MEM), Aztreonam (ATM), Amoxicillin/Clavulanic acid (AMC), Cefixime (CEF), Cefuroxime (CXM), Ceftriaxone (CRO), Cephalothin (CE), Amikacin (AK), Gentamycin (CN), Tetracycline (TE), Ciprofloxacin (CIP), Norfloxacin (NOR), Moxifloxacin (MXF), Septran (SXT), Flucloxacillin (F), Florfenicol (FF), Cefoperazone/Sulbactam (CES), Piperacillin/Tazobactam (TPZ), Colistin (CT), Chloramphenicol (C), Tobramycin (TOB), Cefepime (FEP) and Levofloxacin (LEV). Results were interpreted based on the appearance of the zone of inhibition and no zone of inhibition around discs. The microbiological data of *Acinetobacter* pre-COVID-19 and during COVID-19 were analyzed using the SPSS (Statistical Package for Social Sciences) version 26.0 software. Descriptive statistics were premeditated for all variables. Definite data were concise, including incidences and proportions. The Chi-square test calculated the changes between antibiotic resistance values pre and during COVID-19, and p-values ≤ 0.05 were considered statistically significant.

RESULTS

In this study, the data were evaluated in two different periods: Before COVID-19 (January 2017-December 2019) and during the COVID-19 period (January 2020 to December 2020). A total of 625 strains of *Acinetobacter* specie were collected from different divisions of Punjab province, 462 (73.9%) patients in the pre-COVID-19 period and 163 (26.0%) patients during the COVID-19 period from both genders,

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percentage of females in pre COVID-19 and COVID-19 era were 53%. The proportion of males was 46% and 45% respectively, as shown in table 1.

Table 1: Gender-Based Distribution in Pre-COVID-19 and During the COVID-19 Era

Gender	Pre COVID-19 N (%)	During COVID-19 N (%)	p-Value
Total (n=625)	462 (74)	163 (26)	<0.000
Male	198 (46)	92 (45)	0.825
Female	231 (53)	104 (53)	1

Distribution of *Acinetobacter spp* among different specimen types like pus (47%), urine (21%), sputum (7%), high vaginal swab (HVS)(6%), tracheal secretion, bronchial wash (5%), blood (4%), tips (4%), semen (2%), tissue (2%), Cerebrospinal Fluid (CSF)(1%) and other body fluids (1%) in pre-COVID-19 while in COVID-19 period pus (48%), urine (16%), sputum (5%), High Vaginal Swab (HVS)(1%), tracheal secretion, bronchial wash (6%), blood (8%), tips (6%), semen (1%), tissue (2%), Cerebrospinal Fluid (CSF)(3%) and other body fluids (4%). Mainly, it was collected from pus, followed by urine and blood, as shown in table 2.

Table 2: Distribution of *Acinetobacter spp* Among Different Clinical Samples in the Pre and During COVID-19 Period

Gender	Pre COVID-19 N (%)	During COVID-19 N (%)	p-Value
Pus	215 (47)	79 (48)	0.826
Urine	96 (21)	26 (16)	0.167
Sputum	33 (7)	8 (5)	0.372
HVS	27 (6)	2 (1)	0.009
T Secretion/B Washing	23 (5)	9 (6)	0.622
Blood	20 (4)	13 (8)	0.045
Tip	18 (4)	9 (6)	0.291
Semen	10 (2)	1 (1)	0.401
Tissue	8 (2)	4 (2)	0.89
CSF	6 (1)	5 (3)	0.073
Body Fluids	6 (1)	7 (4)	0.012

The age group which was mainly infected was 16-30 years (34%) followed by the age group of 31-45 years (27%), as shown in table 3.

Table 3: Different Age Groups Having Acinetobacter Infection

Groups	Up to 15 Years N (%)	16-30 Years N (%)	31-45 Years N (%)	46-60 Years N (%)	> 60 Years N (%)
Pre COVID	42 (9)	157 (34)	124 (27)	84 (18)	55 (12)
During COVID	22 (13)	55 (34)	43 (26)	28 (17)	15 (9)

The following drugs showed a substantial increase in resistance during the COVID-19 period: IMP 53% to 63%, ATM 91% to 100%, CIP 65% to 75%, MXF 66% to 100%, CES 61% to 97% and TPZ 61% to 71% as shown in table 4.

Table 4: Different antibiotic drugs that were used in susceptibility testing in the pre-COVID and during the COVID-19 period[a]

Name of Antibiotics	Pre COVID-19 N (%)	During COVID-19 N (%)	P-value[b]
Ampicillin (AMP)	96	100	0.009
Imipenem (IMP)	53	63	0.027
Meropenem (MEM)	53	63	0.027
Aztreonam (ATM)	91	100	0.000
Amoxicillin/Clavulanic Acid (AMC)	94	100	0.001
Cefixime (CEF)	98	98	1
Cefuroxime CXM	97	100	0.025
Ceftriaxone CRO	95	97	0.288
Cephalothin (CE)	99	100	0.201
Amikacin (AK)	59	60	0.823
Gentamycin (CN)	69	64	0.241
Tetracycline (TE)	60	54	0.182
Ciprofloxacin (CIP)	65	75	0.019
Norfloxacin (NOR)	19	21	0.58
Moxifloxacin (MXF)	66	100	<0.000
Septran (SXT)	87	94	0.014
Flucloxacillin (F)	25	21	0.304
Florfenicol (FF)	96	100	0.009
Cefoperazone/Sulbactam (CES)	61	97	<0.000
Piperacillin/Tazobactam (TPZ)	61	71	0.022
Colistin (CT)	1	2	0.325
Chloramphenicol ©	74	74	1
Tobramycin (TOB)	54	37	0.000
Cefepime (FEP)	94	96	0.335
Levofloxacin (LEV)	64	64	1

[a] results are reported as % unless otherwise indicated
[b] chi-Square

DISCUSSION

Acinetobacter is an organism with a complex genus, and factually, there has been misperception about the presence of various species. *Acinetobacter spp* are usually the source of many infections like hospital-acquired infections, folly catheter-associated bacteremia, pneumonia, urinary tract infections, bone infections, soft tissue infections, and central nervous system disease [4]. Similar to another study, *Acinetobacter spp* was resistant to most antimicrobials other than colistin [25]. Antimicrobial resistance is the primary cause of death worldwide; almost 1.27 million deaths occurred statistically due to acquiring resistance against bacterial agents in 2019 [26]. Internationally, 700,000 deaths were estimated due to drug-resistant infections annually [20]. During the COVID-19 pandemic, severe socio-economic losses affected the transmission and epidemiology of different bacterial infections. The overuse of antibiotics with different combinations has led to a silent outbreak of antibiotic resistance that increased during the COVID-19 pandemic. This might adversely influence AMR, particularly in several

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developing countries that already recognized the occurrence of MDR bacteria before the pandemic [19]. It is undecided whether the penalties of the practices that followed in the COVID-19 pandemic positively or negatively affected the proportions of AMR [27]. This retrospective study was used to conclude the difference between the antimicrobial resistance of *Acinetobacter spp* from different divisions of Punjab before and during the COVID-19 outbreak. The existing research comprises different clinical samples from where Acinetobacter was isolated; pus and urine specimens had a higher percentage than other samples like blood, sputum, and body fluids in both the pre and COVID-19 eras [28]. Total 625 *Acinetobacter spp* were collected, 462 (74%) from COVID-19 and 163 (26%) during the COVID-19 era, respectively. Generally, antimicrobial resistance rates were high in both periods. Approximately 25 antimicrobials in the current study were tested, and most of them presented higher resistance during the COVID-19 period than in the pre COVID-19 period. In this study, females (53%) were more prone to infection with *Acinetobacter* than males (45%) in both eras. Similarly, the age group most affected by infection was 16-30 years. Antimicrobial resistance of Acinetobacter against Amikacin, Ciprofloxacin, Cotrimoxazole, and Piperacillin-Tazobactam 59%, 65%, 87%, 61% in COVID-19 and during the COVID-19 era 60%, 75%, 94%, and 71% was noted in this study which was not much similar according to Saini *et al.,* 2021 60%, 80%, 64%, 84% (pre COVID-19) and 80%, 80%, 87% and 94% (during COVID-19). Both studies showed that different proportions increased resistance against these antibiotics during COVID-19. These differences exist because the strains of *Acinetobacter* included by Saini V *et al.,* were low in number compared to our study [29]. Another survey by López-Jácome LE *et al.,* in 2022 noted AK, AMC, CIP, IMP, MEM, TAZ, SXT resistance rates were increased during the COVID-19 period, which was similar to our study that was AK 60%, acid AMC 100%, CIP 75%, IMP 63%, MEM 63%, TAZ 71% and SXT 94% [29]. Other antibiotics like gentamicin, tetracycline, tobramycin, and nitrofurantoin resistance decreased during COVID-19. Usage of these antibiotics during COVID-19 may be less because gentamicin and tobramycin affect kidneys. Other drugs like nitrofurantoin and tetracycline are mainly used to treat urinary tract infections. The Cephalosporin group has no significant difference in pre-COVID-19 and during the COVID-19 period.

CONCLUSIONS

The existing study explored the position of Antimicrobial Resistance (AMR) profiles of *Acinetobacter spp.* They were isolated in the pre and during COVID-19 era from different divisions of Punjab Lahore. It was detected that there was a significant rise in the resistance to various antimicrobial drugs during the COVID-19 era compared to the pre-COVID-19 period. In the COVID-19 pandemic, the abandoned use of broad-spectrum antimicrobial drugs to treat *Acinetobacter* infection led to resistance. Data from the COVID-19 pandemic showed the resistance rates of antibiotics like Amikacin, Amoxicillin/Clavulanic Acid, Ciprofloxacin, Imipenem, Meropenem, Piperacillin/Tazobactam, Aztreonam, Moxifloxacin, Sulzone and Trimethoprim/Sulfamethoxazole was significantly more than pre COVID-19. The resistance rates of Gentamycin, Tobramycin, Tetracycline, and Nitrofurantoin decreased during the COVID-19 pandemic. It has been observed from the current study that resistance to most antibiotic drugs was significantly increased, and they may be frequently used in private community clinics and hospital settings by local community pharmacies.

Authors Contribution

Conceptualization: MA
Methodology: ST, KN, HB
Formal analysis: SS, SR
Writing, review and editing: MA, ST, KN

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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