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ACKNOWLEDGEMENT

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Peptides and Cancer

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Peptides exert oncogenic or anticancer effects on many cancer types and, compared with normal cells, cancer cells overexpress peptide receptors. Overexpression of the peptidergic systems (peptides and/or peptide receptors) by tumors has been related with tumor size, relapse risk, aggressiveness, and poor prognosis. This overexpression serves as tumor biomarker for diagnosis/treatment and opens the door to use compounds favoring a more specific destruction of cancer cells than the anticancer strategies currently used in clinical practice. Oncogenic peptides favor the proliferation, invasion, migration and metastasis of tumor cells, exert an anti-apoptotic action in these cells, and promote lymphangiogenesis and angiogenesis, therefore favoring tumor development, whereas peptide receptor antagonists counteract all the previous oncogenic effects [1]. Different tumor types overexpress the same peptide receptor and this suggests that the same antitumor strategy (e.g., peptide receptor antagonists, peptide receptor radionuclide therapy, cytotoxic peptide conjugatebased cancer therapy) could be applied to treat different cancers. Anticancer peptides exert antiproliferative, apoptotic and antimigration actions in cancer cells as well as anti-angiogenic effects; that is anticancer peptides and peptide receptor antagonists exert the same antitumor actions. Peptides receptor antagonists show a high therapeutic capacity because peptides/peptide receptor agonists have poor bioavailability and short half-life, although the latter compounds have a higher solubility and safety. However, many strategies to increase the therapeutic effect of peptides, their delivery and stability have been developed [2]. Peptides and peptide receptor antagonists are therapeutic tools to fight cancer and hence ligands and receptors of the peptidergic systems open the door to develop new, specific and promising anticancer strategies. In fact, the Food and Drug Administration (FDA) has approved gonadotropin-releasing hormone receptor agonists to treat breast and prostate cancers and somatostatin peptide analogs for diagnostic and treatment purposes in tumors expressing somatostatin receptors (e.g., lung, neuroendocrine) [2]. Moreover, the repurposing of the antiemetic drug aprepitant (a neurokinin-1 receptor antagonist) as anticancer agent has been suggested [3]. This confirms the important roles that peptidergic systems play in cancer progression and how the current knowledge on these systems can be applied in clinical practice because peptidergic systems are crucial therapeutic targets for cancer treatment [4].

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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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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

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.

> 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

Arif M et al.,

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 immunecompromised 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 Chisquare test calculated the changes between antibiotic resistance values pre and during COVID-19, and p-values \leq 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,

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 \mbox{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 susceptibilitytesting in the pre-COVID and during the COVID-19 period^a

	Pre COVID-19	During COVID-19	P-
Name of Antibiotics	N(%)	N(%)	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

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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Original Article

Prevalence of Varicose Veins and Quality of Life among Security Guards

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INTRODUCTION

Varicose veins are a condition affecting the lower limbs, characterized by the distension and swelling of veins due to the malfunction or failure of the valves within them. This leads to blood backflow and accumulation in the limbs. Superficial thrombophlebitis may develop, potentially causing prolonged bleeding. Patients with varicose veins often experience tenderness due to venous distension [1]. Female-to-male ratio of varicose veins is 3:1. However, other studies have reported a higher prevalence in males. Varicose veins are more common in developed and industrialized countries compared to underdeveloped ones[2]. Common symptoms include visible twisted veins, leg cramps, heaviness, swelling, pain, and fatigue [3]. In

ABSTRACT

Varicose vein is a condition of lower limbs which is caused by distension and swelling of veins leading towards back flow and accumulation of blood in limbs. Objective: To find out the prevalence of varicose veins and quality of life among the security guards of Lahore. Methods: Data were collected from the 210 security guards of public areas of Lahore, using convenient sampling. Both male and female security guards 35-70 years with at least 6 duty hours and having more than 1 year of experience were included while; security guards with chronic diseases, history of trauma and amputated lower limbs were excluded. Perthe's test and Brodie-Trendelenburg Test were applied to rule out the VV and Aberdeen's Varicose Veins Questionnaire (AVVQ) was used to evaluate the quality of life. SPSS 26.0 was used to compute the results. Results: Mean age of the security guards was 44.661 ± 7.315 years. Females were 8(3.8%) and males were 202 (96.2%). Majority 204 (97.1%)) of participants had 7 to 10 duty hours. Brodie-Trendelenburg test shows positive response in 18(8.6%) participants for their right leg and 16 (7.6%) for left leg. While, Perthes test was positive in 16(7.6%) participants for left leg, whereas 17 (8.1%) for right leg. Prevalence of varicose veins in security guards was found 8%. Majority of participants 199(94.8%) presented good quality of life while, 11(5.2%) reflected moderate quality of life. Conclusions: The prevalence of varicose veins among security guards of Lahore was low. The quality of life was good in the majority of the security guards.

2022, Aslam MR *et al.*, conducted a global study on the development of varicose veins, including its epidemiology and contributing factors. They found that the prevalence of varicose veins varies worldwide, with a rate of 16-20% in Pakistan [4]. Risk factors for varicose veins include age, gender, family history, occupation, pregnancy, Body Mass Index(BMI), and obesity. People over 65, women, those with a family history of the condition, individuals in long-standing occupations like teaching and security, pregnant women, and those with higher BMI or obesity are at increased risk [4]. Other high-risk groups include the elderly, overweight individuals, those lifting heavy objects, pregnant women or those at menopause, and people

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consuming low dietary fiber [5]. Security guards are particularly prone to varicose veins due to prolonged standing hours and higher BMI. Signs of varicose veins include spider veins, leg or ankle swelling, lumps in leg veins, skin discoloration, eczema, ulcers, pale ulcers, pain in touchable veins, leg scars, and increased temperature [6]. Doppler ultrasonography is recommended to check for venous reflux. If unavailable, the Brodie-Trendelenburg and Perthe's tests are effective alternatives for diagnosis due to their high sensitivity [7-9]. Security guards, who often belong to low-income professions and endure prolonged standing, may not prioritize their health until it becomes a serious issue.

Our study focused on examining the prevalence of varicose veins among security guards and how this condition affects their quality of life. This information could be useful in developing a prognostic criterion before the condition becomes severe. The objective of the study was to determine the prevalence of varicose veins and assess the quality of life among security guards in Lahore.

METHODS

This Descriptive cross-sectional study was conducted after ethical permission reference no. (Case no. 626/ERC/CMH/LMC) in four months (September to December 2023), on the security guards of public areas (universities, banks, schools, markets and hospitals) of Lahore. The sample size was estimated using WHO calculator, utilizing the findings of a previous study with 95% confidence interval and was found 210 participants [10]. Non-probability convenient sampling technique was used to meet the sample size. Companies providing security guards to different institutions was formally approached and with their assistance the security guards were approached at their duty sites of both gender ranging from age 35-70 years, having at least 6 hours per day duty timings and having more than 1 year of experience as a security guard were included while, security guards with other chronic diseases, with the history of trauma or with amputated lower limbs, pregnant females or postmenopausal lady guards were excluded. Before the data collection consent were taken and interview was conducted after explaining the whole procedure. Varicose Veins were subjectively confirmed by application of Perthe's Test (Sensitivity 0.97% and Specificity 0.20%) and Brodie Trendelenburg Test (1) (Sensitivity 0.91% and Specificity 0.15%) (Figures 1: A& B). The Brodie-Trendelenburg test is useful for diagnosing varicose veins

and determining whether the reflux is superficial or deep. The test involves having the patient lie down and elevate their leg to empty the veins. A tourniquet is then applied, and the filling time is observed when the patient stands up [1]. Quality of life was studied using Aberdeen's Varicose Veins Questionnaire (AVVQ) which has a high test (99%) and Retest (97%) validity [11]. AVVQ is a 13-item questionnaire, its score ranging from 0 to 100 points, with 0 points indicating the best possible quality of life. The data were entered through SPSS version 26.0 and descriptive analysis for baseline and demographic characteristics was done. Covariate analysis for association between Perthe's test, brodie-Trendelenburg test and BMI and quality of life on the basis of scores of AVVQ was done through chi square test($p \le 0.05$ as significant).



Figure 1: Perthes Test Performance and Brodie Trendelenburg Test Performance

RESULTS

The mean age of security guards was $44.66 \pm 7.316.133$ (63.3%) security guards had a normal BMI. 204 (97%) security guards working for 7-12 duty hours. Demographics including gender, age, working experience in years, Body Mass Index (BMI) and duty hours were given as table 1. Results of Perthe's test and Brodie-Trendelenberg's test were given as figure 2. The quality of life on the basis of scoring of Aberdeen's Varicose Vein Questionnaire (AVVQ) was good in 83.33% of security guards.

Table 1: Demographics Characteristics of the Participants (n=	210)
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Variables	Categories	N (%)					
Gender							
Male	-	202(96.19%)					
Female	-	8(3.81%)					
	Age Group (Years)	•					
Mean ± SD	44.66 ± 7.316						
Min-Max	35-65 Years						
	35-40	72 (34.3)					
	41-45	72 (34.3)					
Dongo	46-50	28(13.3)					
Range	51-55	49(23.3)					
	55-60	13(6.2)					
	61-65	1(0.5)					
	01-5 Years	103 (49.0)					
Working	06-10 Years	65 (31.0)					
Experience	11-15 Years	17(8.1)					
(Years)	16-20 Years	13(6.2)					
	>20	12 (5.7)					
	Underweight (<18.5)	7(3.3)					
BMI	Normal weight (18.5-24.9)	133 (63.3)					
(Kg/m²)	Overweight (25-29.9)	56(26.7)					
	Obesity(>30)	14 (6.7)					
Duty Houro	1-6	6(2.9)					
Duty Hours	7-12	204 (97.1)					
	Total	210 (100)					

Figure 2 showed that when performing Brodie-Trendelenburg test, 192 (91.4%) security guards showed negative results while 18 (8.6%) showed positive result for right leg. In Perthes test, 193 (91.9%) security guards showed negative results while 17(8.1%) show positive result for right leg. 194 (92.4%) security guards show negative results, while 16 (7.6%) security guards show positive results for both-Trendelenburg test and Perthes in left leg. **DOI:** https://doi.org/10.54393/pbmj.v7i6.1113



Figure 2: Results of Physical Test of Both Legs in Security Guards In table 2, the positive response for Brodie-Trendelenburg test in right leg, 18 participants out of which 10 (55.6%) participants were of normal weight, 6(33.3%) participants were overweight and 2(11.1%) participants are obese. Their p value is 0.625 and Cramer's V is 0.091. In left leg both Brodie-Trendelenburg and Perthes test showed positive response in 16 (100%) participants, out of which 9 (56.3%) participants were of normal weight,6 (37.5%) was overweight and 1 (6.3%) participant was obese. Their p value is 0.687 and Cramer's V is 0.084. Perthes test of right leg showed negative response in 193 participants and positive response in 17 participants, out of 210 (100%). Positive response was shown in 10 (58.8%) participants with normal weight, 5 (29.4%) overweight participants and 2 (11.8%) obese participants. Their p value is 0.694 and Cramer value is 0.083.

Table 2: Association Between the Brodie-Trendelenburg Test and Perthe's Test in Both Leg and Bmi(Kg/M²)

			BMI (Kg/m²)					p-Value	Cramer's V
Variables	Response	Underweight (<18.5)N(%)	Normal Weight (18.5-24.9) N (%)	Overweight (25–29.9) N (%)	Obesity (>30) N (%)	Total N (%)	N(%)	N (%)	N(%)
Brodie-Trendelenburg	-ve	7(3.6)	123 (64.1)	50(26)	12 (6.3)	192 (100)	1.756	0.625	0.001
Test of Right Leg	+ve	0(0)	10 (55.6)	6(33.3)	2 (11.1)	18 (100)	1.750	0.025	0.091
Brodie-Trendelenburg	-ve	7(3.6)	124 (63.9)	50 (25.8)	13 (6.7)	194 (100)	1.481	0.687	0.084
Test of Left Leg	+ve	0(0)	9 (56.3)	6 (37.5)	1(6.3)	16 (100)		0.007	
Perthe's Test of	-ve	7(3.6)	123 (63.7)	51(26.4)	12(6.2)	193 (100)	1.449	0.694	4 0.083
Right Leg	+ve	0(0)	10 (58.8)	5 (29.4)	2 (11.8)	17 (100)		0.094	0.005
Perthe's Test of	-ve	7(3.6)	124 (63.9)	50 (25.8)	13 (6.7)	194 (100)	1 / 01	0.007	0.084
Left Leg	+ve	0(0)	9 (56.3)	6 (37.5)	1(6.3)	16 (100)	1.481	0.687	0.084
Total		7(3.3)	133 (63.3)	56 (26.7)	14 (6.7)	210 (100)	-	-	-

In table 3, Brodie-Trendelenburg test of right leg showed r value of 0.462 with a positive response in 18 participants out of 210 (100%) where 11 (61.1%) and 7 (38.9%) participants belonging to groups <34 (good) and 34-66 (moderate) quality of life respectively. The Brodie-Trendelenburg test and Perthe's test in left leg is in 7(43.8%) and 9(56.3%) participants belonging to groups <34 (good) and 34-66 (moderate) quality of life respectively in 16 participants out of 210 (100%), who showed up with positive results. Their r value is 0.658. Perthe's test of right leg showed r value of 0.4 with a positive response in total 17

<0.001*

<0.001*

_

Spearman Correlation Coefficient(r)

0.462

0.658

0.401

0.658

_

participants, where 11(64.7%) and 6(35.3%) belonged to groups <34(good) and 34-66(moderate) quality of life respectively. P value is significant for both tests in both legs.

9 (56.3)

5(2.6)

6(35.3)

2(1)

9 (56.3)

11(5.2)

16 (100)

193 (100)

17(100)

194 (100)

16(100)

210 (100)

33.504

90.797

_

		Quali	Quality of Life		chi-	
Variables	Response	<34 (Good) N (%)	34-66 (Moderate) N (%)	Total N (%)	Square	p- Value
Brodie-Trendelenburg	-ve	188 (97.9)	4 (2.1)	192 (100)	44.913	<0.001*
Test of Right Leg	+ve	11 (61.1)	7(38.9)	18 (100)	44.915	<0.001
Brodie-Trendelenburg	-ve	192 (99)	2 (1)	194 (100)	90,797	<0.001*
Test of Left Lea	11/0		0 (50.7)	10(100)	1 90./9/	<0.001

Table 3: Association Between Brodie-Trendelenburg Test and Perthe's Test with Quality of Life

7(43.8)

188 (97.4)

11(64.7) 192 (99)

7(43.8)

199 (94.8)

+ve

-ve

+ve

-ve

+ve

DISCUSSION

Total

Test of Left Leg

Perthe's Test of

Right Leg

Perthe's Test of

Left Leg

The prevalence of the varicose veins in security guards is low. Ramyashree, et al., in 2022 conducted research about knowledge of varicose veins among 73 guards in a selected hospital in Mangalore India, where majority of the security guards (46.6%) were above 41 years, while 93% of them were males. The service years of majority these security guards were 1-5 years, while majority (83.6%) were standing during work and their working hours was 8 to 12 hours. However, no association was found between their knowledge and the selected demographic variables [12]. Most of current study's findings are similar to them with a difference in sample size. Moreover, the ratio of females was also low likewise the current study. A similar study was conducted in Bengaluru Karnataka, to assess the risk factors among traffic police personnel by Mohiddina DF et al., their sample included only 50 members and 60% of them had prolonged working hours, 38% of the sample population belonged to 50-59 years' age group. Though females are more vulnerable to develop VV than males owing to hormonal factors, but the present study involves only 2% female population, rest 98% being males [13]. The male to female ratio in both studies is almost comparable but their sample was smaller and population was different. Timilsina R et al., conducted a cross-sectional study on the assessment of risk for varicose veins among city police working in Belagavi city, Karnataka, in 2021. The participants provided clear consent and were thoroughly interviewed with the help of a questionnaire and observational techniques to determine the signs and symptoms. The study concluded that 14.7% of the police officers had varicose veins which showed an obvious association with their religion, designation, duration of working, income, marital status, lungs problem, DVT, cardiac infarct, and HTN. In association with marital status and prolonged standing, 20% of the participants were found at risk of developing varicose veins in their later age. Other possibilities due to which varicose veins may develop

are a diet that contain low fiber, genetically weaken vein walls, aging and females after menopause are at greater risk to get varicose veins. VV's can be prevented by raising the leg above the heart level and this should be done for quite a few minutes in a day. The COVID pandemic and ongoing election campaign at that time of study did not allow the researchers to perform the Doppler US due to which they missed first-stage cases of varicose veins [14]. The similarity is being the common limitation of not using the colored doppler US due to different reasons. It is seen that the prevalence of varicose veins varies with the side of the limb being examined. Two physical tests were performed on both limbs of all the individuals to assess the prevalence of the varicose vein. Out of 210 security guards, the Brodie-Trendelenburg test for right leg came out negative for majority (192) of the individuals with mean SD of 2.05 ± 8.45 with a t value -5.338 and p value is >0.001, while for the left leg it was 194 out of 210 with mean SD of 1.87 ± 7.56 with a t value -6.227 and p value is >0.001. Right leg has a higher number of positive tests results as compared to the left leg. Alghamdi DA et al., conducted a study in 2020 to elaborate the effects of varicose veins on quality of life of adult female patients in the Eastern region of Saudi Arabia. 128 female patients were included in the study that were aged 18 years or above. The majority (44.5%) of them were between the age group of 45 to 54 years. The body mass index ($\beta = -0.304$, t=-2.870, p=0.005), frequent constipation (β =-0.258, t=-2.870, p=0.009), and long rest periods during work (β =0.517, t=2.111, p=0.037) came out to be important predictors of quality of life [15]. The difference is that they included only females and they observed effects of VV on QOL to determine potential associations between demographic characteristics of these patients and their Quality of life, while in current study male population was dominant. Another study by Branisteanu DE et al., conducted on 1893 Romanian population, confirms that BMI was associated with the

severity of chronic venous disease for the female group in their study [16]. This finding validates the possible explanation of the poor QoL among obese individuals, as they may suffer from a more severe form of the disease. Ali AL et al., conducted a study in 2019 to evaluate the prevalence of varicose veins among secondary schools teachers. The findings of this study revealed that mean age of studied sample were 45.11 ± 9.09 and 41.4% of participants age ranged from 35-44 years. Compared to current study where the mean age was 44 and average age group ranged from 35-40 years, this study is quite similar to ours in demographics. The outcome of the study was a high prevalence of varicose veins among secondary schools' teacher while our study showed low prevalence of varicose veins among security guards [17]. The current study has shown association of the quality of life with prevalence of varicose veins in both sides of limbs after performing Brodie-Trendelenburg and Perthes test. According to the study, 192 were tested negative for Brodie-Trendelenburg test in the right leg. In these 192 individuals, 188 individuals have good quality of life while 4 individuals indicated moderate quality of life with a p value=0.001 and r value=0.462. For the positive participants.in Brodie-Trendelenburg test, the quality of life was good for 11 individuals and moderate for 7, out of 18 individuals in total. The number was quite same for the left leg of Brodie's Trendelenburg test members. The test results came out negative for 194 individuals out of which 192 had good quality of life while 2 had moderate quality of life with a p value=0.001 and r value=0.658. The test came positive for 16 members, out of which 7 had good quality of life and only 9 people had moderate quality of life. After evaluating the association of quality of life with Perthe's test, we can see that for the right leg 193 out of 210 members came out negative result with a p value=0.001 and r value=0.4. Out of 193 individuals, 188 members had good guality of life while 5 have shown moderate quality of life. For the positive members of the test, 11 out of 17 members have good quality of life while 6 had moderate quality of life. Similarly, in the left leg, 194 members came out negative out of which 192 had good quality of life and the remaining 2 had moderate quality of life with p=0.001 and r value=0.658. For the positive members of the Perthes test in the left leg, 7 out of 16 members had good quality of life while the other 9 had moderate quality of life. The above statistics indicate that quality of life is associated with prevalence of varicose veins as the members with positive test results of both tests in both limbs tend to 42 have moderate quality of life as compared to the negative members who have good quality of life. A similar study was conducted by Tan MK, et al., in year 2019 to assess the relationship between the diameter of the veins, clinical severity and quality of life. It was systematic review that included 9 cross sectional studies and a prospective cohort study. The available evidence suggests that while truncal venous diameters

have a weak positive correlation to clinical severity described using the CEAP classification or VCSS, they hold no relationship or predictive value of patients' HRQoL. This is in contrast to the significant relationships between clinical severity and HRQoL, with most studies showing correlation between clinical severity classification systems [18]. In an occupational setting, prolonged standing is closely associated with varicose veins. The workers most commonly affected are nurses, hairdressers, clerks, security guards, physical laborers, etc. [19]. The Clinical, Etiological, Anatomical, and Pathological CEAP classification divides varicose veins into seven clinical stages, ranging from CO to C6, and is abbreviated as Clinical, Etiological, Anatomical, and Pathological Elements [20]. G. Aly S et al., in Cairo conducted a crosssectional study on prevalence and associated risk factors of varicose vein among women with mean age 35.84 ± 9.14 years and standing hours during working days were 7.72 ± 2.54 days while in our study mean age was 44.66 ± 7.31 and duty hours were 1.97 ± 0.16 hours. In their study, 51.1%females were diagnosed with varicose veins by clinical examination and questionnaire while 8.1% security guards were diagnosed with varicose veins by physical tests and AVVQ. It shows women are at high risk of getting varicose veins rather than males [21]. Abou-ElWafa HS et al., conducted a cross sectional descriptive study among 201 nurses, specifically at a university in Egypt, showing prevalence of 18.4%. While our study conducted in public areas (hospital, office, market, bank) showing 8.1% prevalence among 210 security guards. Both studies show people having more duty hours are more prone to develop varicose veins [22]. Al Bader B et al., in 2020) found low prevalence in nurses in Riyadh [23]. Due to non-flexible duty hours of the security guards, it was challenging for researchers to access them thoroughly. Tests utilized for assessment cannot be conducted in open public areas and need a facility. The unavailability of a proper setting (cabins, coaches) for the performance of Brodie-Trendelenburg and Perthes test also came out as a limitation while conducting the research. Color Doppler is a gold standard tool for the diagnosis of VV, but due to financial and ethical limitations, researchers could not utilize that investigation for the study population. The importance of the study is that, with this investigation, many of the participants became aware of their health risks and requested for advice on how to prevent it from further progression.

CONCLUSIONS

The prevalence of varicose veins among security guards of Lahore was low. The quality of life was good in the majority of the security guards of Lahore.

Authors Contribution

Conceptualization: SA, HQ, WP, RA Methodology: SA, HQ, RA, MBA, MH, AR Formal analysis: AR Writing, review and editing: SA, HQ, WP, RA

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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Original Article

Evaluating Emotional Intelligence Among Undergraduate Medical Students: A Cross-Sectional Study from Peshawar, Pakistan

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ABSTRACT

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INTRODUCTION

An individual's capacity to comprehend and respond to their own and others' emotions, differentiate among them, and use this skill in directing their thoughts and actions is defined as Emotional Intelligence as defined by Salovey and Mayer [1, 2]. It relates to many parts of medical practice, such as diagnostics, doctor-patient relationships, teamwork (doctor-doctor relationship), interaction, and compassion, and can thus be utilized to enhance both the therapeutic and academic components of medicine[3]. It was found that those with high emotional intelligence experienced less stress, were in better health

An individual's capacity to comprehend and respond to their own and others' emotions, differentiate among them, and use this skill in directing their thoughts and actions is defined as Emotional Intelligence. Objective: To evaluate the emotional intelligence (EI) of undergraduate medical students in Peshawar, Pakistan, based on their gender and year of study. Methods: This research project used a cross-sectional, observational study design. The study was carried out in public and private sector medical colleges in Peshawar, Pakistan, from August 2023 to December 2023. A total of 525 complete answers were received. Schutte's Self-Report Emotional Intelligence Test (SSEIT) was the tool utilized to gather data. The data were analyzed using SPSS version 29.0. Results: A mean age of 21.12 ± 1.754 was observed among the participants, whose ages varied from a minimum 17 to maximum 28. The overall participant's mean emotional intelligence score is 116.62 14.948. Male scored higher on emotional intelligence (117.36) than female (116.30) and both genders were statistically significant. Compared to clinical science students (115.18), basic science students (117.32) had a higher emotional intelligence mean score, whereas there was no statistical significance between the two groups. Conclusions: The research concluded that the majority of undergraduates had an average degree of emotional intelligence, with men scoring higher than women. Also, students studying basic sciences had higher mean scores than clinical science students.

> due to their ability to control their emotions, were able to adjust to their environment, and excelled academically as well as socially [4, 5]. Hence empathic clinicians are more likely to increase patient-doctor relationships, patient trust, contentment, and compliance, as well as generate better therapeutic outcomes [6, 7] It has also been proposed that self-efficacy is related to El, which is described as having faith in one's capacity to perform well at tasks. Furthermore, successful leadership requires self-awareness, self-management, motivation, empathy, and interpersonal abilities [8]. Compared to earlier scores,

research has indicated a rise in patient satisfaction after training in emotional intelligence for medical residents [9]. Anxiety affects nearly one-third of medical students, according to a recent meta-analysis, it has risen to become the world's sixth biggest cause of disability. Because of its high frequency, major illness load, and detrimental consequences on academic performance, quality of life, professionalism, and patient care quality, anxiety in medical students warrants further attention. El may be a key predictor of anxiety, according to empirical research [10]. Lately, there has been a push to integrate EI training for healthcare professionals to strengthen leadership, skills to communicate, and minimize stress and burnout. The El abilities offer a foundation for residents and students to achieve effectiveness. Therefore, the first step in successfully utilizing an El approach in medicine is evaluating El in individuals [11]. As El impacts medical education and practice tremendously, it is of paramount importance to evaluate undergraduate medical students' current levels in this respect.

This study aimed to address the knowledge gaps by providing estimates of El in this demographic group and investigating the variables of gender and academic discipline; therefore, provides information on interventions and training that could benefit emotional and professional competencies in prospective doctors.

METHODS

This research project used a cross-sectional, observational study design. The study was carried out in public and private sector medical colleges in Peshawar, Pakistan, over five months from August 2023 to December 2023. For this study, non-probability convenient sampling was used. 471 was the sample size with a 97% confidence level that was determined using openepi.com. Google forms and questionnaires were dispersed to several medical colleges. A total of 525 complete answers were received, of which 351 came from private medical institutions and 174 from public medical colleges. Voluntary consent from students enrolled in undergraduate programs served as the study's inclusion criterion. Students who were unwilling to participate, those who dropped out, those who graduated, and interns were all excluded. Incomplete questionnaires were also a reason for exclusion. Every participant received a thorough explanation of the goals and purposes of the study, and consent was obtained. The project was approved by the Northwest School of Medicine's Institutional Review Board and Ethics Committee (IRB&EC/2023-SM/065) Schutte's Self-Report Emotional Intelligence Test (SSEIT) was the tool utilized to gather data [12]. The 33 items on the test examine six different dimensions: positive affect, other people's emotions, happiness, one's own emotions, nonverbal emotions, and emotional management. Except for three variables (i.e., 5, 28, and 33) that were reverse

scored, all 33 items were based on a 5-point Likert scale, where 1 represents strongly disagree and 5 represents strongly agree. The highest score on the scale indicated a high level of emotional intelligence. The scores varied from 33 to 165. Individuals scoring 77 or lower were classified as having low emotional intelligence, those scoring 78 to 121 as having average emotional intelligence, and those scoring 122 to 165 as having high emotional intelligence. The demographic factors included in the questionnaire are the following: age, gender, study year, and institution. Students in their first and second years were placed in the basic science category, while those in their third, fourth, and final years were placed in the clinical science category. The data were analyzed using SPSS version 29.0, frequency analysis and descriptive statistics were utilized. The Independent Sample t-Test was also utilized to determine whether there was a significant difference between the genders of the male and female students as well as between basic and clinical science students.

RESULTS

Out of the 525 participants in the study, 33.1% were affiliated with public medical institutions in Peshawar, Pakistan, while 66.9% were affiliated with private institutions. Participants ranged in age from minimum 17 years to maximum 28 years, with a mean age of 21.12 ± 1.754 . Of the participants, 157(or 29.9%) were men and 368(70.1%) were women(Table 1).

Table 1: Demographics Characteristics of the Participants (n=210)

Variables	Male (%)	Female (%)	Total (%)			
Institution of the Participants						
Public	2 (1.1)	172 (98.9)	174 (100)			
Private	155 (44.2)	196 (55.8)	351 (100)			
Year of Study of the Participants						
First Year	105 (47.7)	115 (52.3)	220 (100)			
Second Year	20 (14.9)	114 (85.1)	134 (100)			
Third Year	5(11.6)	38(88.4)	43 (100)			
Fourth Year	8(10.8)	66(89.2)	74 (100)			
Final Year	19(35.2)	35 (64.8)	54 (100)			
Total	157(29.9)	368 (70.1)	525(100)			

Male scored higher on emotional intelligence (117.36) than female (116.30). Additionally, there existed a statistically significant difference between the two genders (Table 2).

Table 2: Comparison of Emotional Intelligence Score with Gender
of the Participants (Independent Sample t-test)

Gender	Frequency (%)	Mean ± S.D	p-Value
Male	157 (29.9)	117.36 ± 16.801	0.017
Female	368 (70.1)	116.30 ± 14.096	0.017

Compared to clinical science students (115.18), basic science students (117.32) had a higher emotional intelligence mean score, however, there was no statistically significant difference (Table 3).

Table 3: Comparison of Emotional Intelligence Score with theYear of Study of Participants (Independent Sample t-test)

Year of Study	Frequency (%)	Mean ± S.D	p-Value	
Basic Sciences Students	354 (67.4)	117.32 ± 14.380	0.063	
Clinical Sciences Students	171(32.5)	115.18 ± 16.007	0.005	

Students' emotional intelligence was divided into three categories: low (1.7%), average (59.8%), and high (38.5%) (Table 4).

Table 4: Levels of Emotional Intelligence of the Participants

Levels	Frequency (%)
Low Emotional Intelligence	9 (1.7)
Average Emotional Intelligence	314 (59.8)
High Emotional Intelligence	202 (38.5)
Total	525(100)

DISCUSSION

Due to its critical importance to both academic achievement and career success, emotional intelligence (EI) has been the subject of increased research over the past 20 years, with numerous academic papers published on the topic [13]. To the best of our knowledge, this is the first study in the Peshawar area to use the Schutte selfreport emotional intelligence test to measure emotional intelligence (EI) in undergraduate medical students. While there was no significant relationship found between gender and emotional intelligence in another study conducted in Iran [3], with study participants consisting of junior and senior medical students, we found a significant difference between emotional intelligence and gender in our investigation. According to a study conducted in Sri Lanka women scored more than men on the El, with a mean score of 241.5[14]. Male participants in our study had mean emotional intelligence scores that were higher than female participants. This indicates that, across the six emotional intelligence variables examined in our study, male individuals outperformed female participants. The mean emotional intelligence score from one institute was 122.4, whereas the score from another institute was 123.3. Our participants' overall mean score came out to be 116.62[11]. It has been demonstrated that empathy, a component of emotional intelligence, improves patient outcomes in healthcare. According to Bertram K. et al., there is a correlation between emotional intelligence and empathy, and women score higher on emotional intelligence and empathy assessments than men do [15]. Medical students frequently experience stress, therefore having strong emotional intelligence can help them manage it. Foster K., reported similar findings in their research on pharmacy, dentistry, and nursing students, where they discovered a negative relationship between perceived stress and emotional intelligence (EI) [16]. Doyle NA et al., likewise noted the same outcomes [17] A further study conducted on Swedish medical students [18] found that those in the

25-29 age range exhibited higher levels of emotional intelligence; this finding may be related to Sweden's later start date for medical schools. The majority of participants (59.8%) exhibited medium emotional intelligence, whereas 38.5% demonstrated high emotional intelligence. Similar findings were noted in a Saudi Arabian study [19], where the majority of pupils (73.4%) had average El. According to Ewaiwe B et al., students studying basic medical science had greater emotional intelligence compared to their clinical medicine counterparts [20]. We saw similar results, with a greater mean emotional intelligence among basic science students. The strengths of the study lie in the large sample size, enhanced reliability and generalizability by using a well-validated data collection instrument (Schutte Self-Report Emotional Intelligence Test) and including public and private sector medical colleges to increase sample diversity. The articulation of the learning processes in terms of different academic years further enables the study of El across various stages of medical education. The limitations include that the data have a subjective bias because they are self-reported and, secondly, the design is cross-sectional; therefore, no causal inferences can be drawn. It is a non-probability sampling by convenience that might reduce generalizability in results, and lastly, there are black-robed cultural variations present at Peshawar, Pakistan, which may impact the finding. It may alter its application from other places.

CONCLUSIONS

It was concluded that the majority of undergraduates had an average degree of emotional intelligence. It was also noted that there was a statistically significant difference in the mean emotional intelligence scores of the genders, with men scoring higher than women. Furthermore, it should be noted that there was no statistically significant difference between the basic science and clinical science groups and that students studying basic sciences had higher mean scores than clinical science students.

Authors Contribution

Conceptualization: SZ, KK Methodology: SZ, KK, MZ, ASK, SM Formal analysis: SZ, KK, MK, KA, MS, AAK, SM, ASK, ZM

Writing-review and editing: SZ, KK, MK, MZ, KA, MS, AAK, ZM

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

Conflicts of Interest

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Original Article

Assessment of Antibiotic Resistance Profiles of Pathogenic Bacteria Isolates from Migratory Birds in the River Ravi Stopover Site

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INTRODUCTION

Migratory birds have been related to the carriage and transmission of antibiotic-resistant microbes that are important for both humans and animals. Every year, billions of birds migrate to practically every continent in the world. Migratory birds, due to their high movement, can acquire and spread antibiotic-resistant pathogens from one location to another [1]. Throughout the migration, they can interact with resident birds, come into encounters with feeds from various settings, and eventually pick up resistant bacteria from those regions. They can operate as reservoirs and propagators of resistant bacteria in aquatic habitats through fecal contamination [2]. *E. coli* [3, 4], *Salmonella spp.* [5], *Staphylococcus* [6], and *Campylobacter*[7, 8] act like the carriers and spreaders by

ABSTRACT

Migratory birds are important vectors for the spread of zoonotic and antimicrobial-resistant (AMR) bacteria, posing a global public health risk. The River Ravi in Pakistan, is a crucial stopover for migratory birds, is seeing increased anthropogenic activity, which contributes to the spread of AMR bacteria. Objective: To assess the prevalence of specific antibiotic-resistant bacterial species among different migratory birds, and significance of migrating birds as possible reservoirs and disseminators of AMR bacteria. Methods: The study was carried out along the Ravi River in Lahore. Fecal samples from five migrating bird species were gathered at five distinct locations utilizing non-invasive mist netting and fresh fecal collection. The bacteria were isolated and identified using Gram staining and biochemical analysis. The Kirby-Bauer technique of disc diffusion was employed to assess the sensitivity of microorganisms. Results: All sites showed significant contamination from Gram-positive and Gram-negative bacteria. Enterococcus spp. and Escherichia coli were most common, indicating fecal contamination. It was found that E. coli and S. aureus are highly resistant to Azithromycin and Gentamycin, whereas K. pneumoniae is resistant to Norfloxacin. However, erythromycin and tetracycline were effective against P. aeruginosa and C. septicum, respectively. Conclusions: The research concluded that the River Ravi was significantly contaminated with germs from humans and the surroundings, along with distinct AMR patterns. The elevated levels of S. aureus, E. coli, Enterococcus spp. and Pseudomonas spp. point to serious health risks for the general public. AMR patterns emphasize the difficulty in controlling bacterial infections, emphasizing the importance of targeted antimicrobial treatments and ongoing surveillance.

> migratory birds. They can be transmitted to people through the consumption of contaminated food and water [9]. Antibiotic use has gradually expanded since its initial use for the treatment of bacterial illnesses. Though antibiotics were first developed to treat humans, they are now widely employed in veterinary care and cattle production. The indiscriminate utilization of antibiotics has led to the development of antibiotic resistance or multidrug resistance in bacteria across several environments. Antibiotic-resistant bacteria obtained in a certain region are simple to spread to other environments, affecting natural ecosystems worldwide. Antibiotic exploitation and malfunctioning healthcare systems have been linked to the proliferation of resistant bacteria in a variety of ecological

factors [10]. The level of antibiotic use influences the number of resistant bacteria in habitat [11]. Remarkably, although not directly linked to human exposures, pristine habitats are implicated in bacterial antibiotic resistance [12]. These findings imply that other factors are influencing the spread of resistant bacteria to such clean habitats. River Ravi is a transnational river that enters Pakistan at Shakkargarh (Sialkot) and falls into River Chenab at Shorkot. The Ravi River supports a variety of habitats, which makes it a significant wintering habitat for migrating birds [13]. However, the area has experienced rapid population growth, the Industrial Revolution, and expansion of agriculture during the last few decades. Human endeavors have increased the consumption of antibiotics while also releasing prescription waste, toxic metals, and other pollutants into the River Ravi ecosystem [14]. Antibiotics along with other selective factors in surroundings can promote the evolution and dissemination of antibiotic-resistant bacteria [15]. The quantity and characteristics of AMR bacteria associated with migrating birds in the River Ravi basin have not been adequately studied, despite the fact that there may be dangers. An effective strategy to curb the spread of drug-resistant germs in the region can only be developed when a thorough comprehension of the role these birds play in AMR transmission is achieved.

The study aimed to assess the prevalence of specific antibiotic-resistant bacterial species among different migratory birds, and significance of migrating birds as possible reservoirs and disseminators of AMR bacteria

METHODS

This study focused on the Ravi river belt in the city of Lahore. Migratory avian species inhabit the vicinity of the Ravi River during winter seasons. The study was conducted between December 2022 and February 2023. The fecal samples were collected from five different locations of the border of River Ravi belt (Talwara) surrounding Lahore [16].





A pilot survey is a small-scale preliminary study aimed at assessing feasibility, with Concerned Department. The meeting was done with Hunter and the Local Community They Consulted with hunters using GPS and other tools for bird tracking and trapping and interacted with local community members including fishermen and residents to facilitate the sampling process. Fresh fecal samples were collected from birds using non-invasive mist nets and fresh fecal sample collection from five sites. The birds were released at their capture sites within 30 minutes to minimize stress and environmental impact. A total of 05 wild bird species (Gad Wall, Pouchard, Teal, Mallard, and Shoveller) were selected for sampling on the basis of availability. In order to detect bacteria from certain bird species, fifty fecal samples were gathered from five different places along the River Ravi belt (Talwara). The fecal matter was collected in sterilized swabs from the birds by mist nets method through cloacal swabbing. It was tried to not the harm or disturbs the birds during sampling. Samples were stored in ice boxes and shifted to the Conservation Biology Lab, Institute of Zoology-University of Punjab of isolation and identification of bacteria. Each swab was streaked on MacConkey agar and incubated aerobically at 35°C for 18-20 hours. Distinct colonies were isolated, purified, and cultured in soft nutrient agar at room temperature for subsequent analysis. The fecal samples, each weighing 1 gram, were diluted in 9 milliliters of normal saline solution, resulting in a series of dilutions ranging from 10^-1 to 10^-10. A 20µl aliquot from each dilution was evenly distributed on nutritional agar plate and subjected to a 24-hour incubation period. Colonies were counted using the formula [17].

CFU/ml= Number of Bacteria colony × Dilution Factor/ Volume of Culture

A sterilized slide was prepared with a saline drop and a single bacterial colony. After drying and heat fixing, slides were stained with crystal violet, iodine, decolorized with acetone alcohol, and counterstained with safranine. Observations under a microscope identified the bacteria as Gram-positive or Gram-negative. The biochemical identification of tests was done through Gram-positive bacteria were tested using the Mannitol Salt Agar (MSA), Catalase and DNase tests. Gram-negative bacteria were detected by employing MacConkey agar and conducting various, tests, including Urease, Motility, Oxidase, Indole, KIA, and TSI assays [18]. The Kirby-Bauer method of disc diffusion was used to measure sensitivity on Muller Hinton agar. The bacterial colonies that were identified were collected using a swab and transferred onto plates. Then, sixteen different antibiotic discs were placed on the plates. These disc included (Cip) ciprofloxacin (5ug), (Nor) norfloxacin (5ug), (Lom) lomefloxacin (5ug), (Tob) tobromycin (10ug), (Cef) ceftriaxone (10ug), (Ste) streptomycine (10ug), (Gen) gentamycin (10ug), (Tri) trimethoprim (25ug), (Tet) tetracycline (30ug), (Dox) doxycen (20ug), (Ery) erythromycine (15ug), (Amp) ampicillin (30ug), (Enr) enrofloxacin (5ug), (Azi) azithromycin (15 µg), (Ofl) Ofloxacin (5µg), (Cep) Cephalexin (10 µg). The plates were then kept in an incubator overnight

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at a temperature of 37°C. The measurement of zones of inhibition was conducted to ascertain the sensitivity of the microorganisms to antimicrobial agents. Data were entered and analyzed by Graph Pad Prism. To create the graphs, OriginPro 2022 was used.

RESULTS

From River Ravi, five different migratory birds' fecal samples were collected through non-invasive method. Gram Positive and Gram Negative bacteria were isolated and identified by a variety of different biochemical tests as described in figure 2.



Figure 2: (a) Non-lactose fermenting colony for Gram Negative bacteria grown on MacConkey agar, (b) Gram Negative bacteria growth (fermenter colonies). (c) Mannitol Salt Agar (MSA) for Staphylococcus aureus (left side of the plate) and *Staphylococcus epidermidis* (right side of the plate). (d) *Salmonella spp.* on SS Agar (e) *E. coli* on EMB Agar (f) AMR Bacteria Drug Sensitivity Test

Gram-positive and Gram-negative bacteria found in the samples are included with the findings of the first sampling site in table 1. The data reflects the microbial variety at the initial sampling site, as some species host numerous types of bacteria.

Table 1: Gram Negative and Gram Positive Bacteria Isolated fromBirds at First Sampling Site

Name of Specie	Gram Negative Bacteria	Gram Positive Bacteria
Gadwall	Salmonella spp.	Staptococus spp.
Pouchard	E. coli spp.	Enterococcus spp.
Teal	E. coli spp.	Enterococcus spp.
Mallard	Pseudomonas spp.	Enterococcus spp.
Shoveller	Pseudomonas spp.	Enterococcus spp.
Gadwall	E. coli spp.	Staphylococcus spp.
Pouchard	E. coli spp.	Staphylococcus aureus spp.
Teal	E. coli Pseudomonas spp	Enterococcus spp.
Mallard	Pseudomonas spp.	Enterococcus spp.
Shoveller	E. coli spp.	Enterococcus spp.

Different bird species and the kinds of Gram-positive and Gram-negative bacteria found in each, along with the results from the second sampling site have been enlisted in

table 2. Gadwall, Pouchard, Teal, Mallard, and Shoveller are among the species that are included. *Pseudomonas species, E. coli species, Klebsiella, Salmonella species,* and *Coliform species* are among the Gram-negative bacteria that were discovered; *Clostridium species, Enterococcus species, Staphylococcus species,* and *Staphylococcus aureus* are among the Gram-positive bacteria that were found. The data demonstrates the microbial diversity found at the second sample site by showing that numerous kinds of bacteria are hosted by them.

Table 2: Gram Negative and Gram Positive Bacteria Isolated fromBirds at Second Sampling Site

Name of Specie	Gram Negative Bacteria	Gram Positive Bacteria				
Gadwall	Pseudomonas spp.	Clostridium spp.				
Pouchard	Pseudomonas spp.	Enterococcus spp.				
Teal	E. coli spp.	Enterococcus spp.				
Mallard	E. coli spp.	Enterococcus spp.				
Shoveller	E. coli spp.	Enterococcus spp.				
Gadwall	E. coli Klebsiella Coliform spp.	Enterococcus Staphylococcus spp.				
Pouchard	E. coli Klebsiella Coliform spp.	Enterococcus Staphylococcus spp.				
Teal	E. coli Salmonella spp.	Staphylococcus aureus				
Mallard	E. coli Salmonella spp.	Staphylococcus aureus				
Shoveller	E. coli Salmonella spp.	Staphylococcus aureus				

The third sampling site yielded a variety of bird species in addition to different kinds of Gram-positive and Gramnegative bacteria, as shown in table 3. The third sampling site's bacterial diversity and prevalence frequency among various bird species are highlighted by this data.

Table 3: Gram Negative and Gram Positive Bacteria Isolated fromBirds at Third Sampling Site

Name of Specie	Gram Negative Bacteria	Gram Positive Bacteria
Gadwall	Salmonella Klebsiella spp.	Enterococcus, Staphylococcus spp.
Pouchard	E.coli	Staphylococcus spp.
Teal	E. coli	Staphylococcus aureus
Mallard	E. coli	Enterococcus spp.
Shoveller	Pseudomonas spp.	Staphylococcus aureus
Gadwall	Pseudomonas spp.	Staphylococcus aureus
Pouchard	E. coli spp.	Enterococcus spp.
Teal	E. coli spp.	Enterococcus spp.
Mallard	E. coli spp.	Enterococcus spp.
Shoveller	Salmonella spp.	Staphylococcus epidermis

The different bird species and the kinds of Gram-positive and Gram-negative bacteria found at the fourth sampling site are shown in table 4. The data presented here illustrates the prevalence frequency and diversity of bacteria across several bird species at the fourth sample site. **Table 4:** Gram Negative and Gram Positive Bacteria Isolated fromBirds at Forth Sampling Site

Name of Specie	Gram Negative Bacteria	Gram Positive Bacteria
Gadwall	Salmonella Klebsiella spp.	Enterococcus, Staphylococcus spp
Pouchard	E.coli	Staphylococcus aureus
Teal	E. coli	Staphylococcus aureus
Mallard	E. coli Pseudomonas spp.	Enterococcus spp.
Shoveller	Pseudomonas spp.	Enterococcus spp.
Gadwall	E.coli	Enterococcus spp.
Pouchard	Pseudomonas spp.	Clostridium spp.
Teal	Pseudomonas spp.	Clostridium spp.
Mallard	Salmonella, Pseudomonas spp.	Salmonella, Pseudomonas spp.
Shoveller	Pseudomonas spp.	Enterococcus spp.

The Gram-positive and Gram-negative bacteria found at the fifth sampling site are listed in table 5 along with a variety of bird species. The data presented here illustrates the prevalence frequency and diversity of bacteria across several bird species at the fifth sample site.

Table 5: Gram Negative and Gram Positive Bacteria Isolated from

 Birds at Fifth Sampling Site

Species of Birds	Gram Negative Bacteria	Gram Positive Bacteria
Gadwall	E.coli, Klebsiella Coliform spp.	Enterococcus, Staphylococcus spp.
Pouchard	E.coli, Klebsiella Coliform spp.	Enterococcus, Staphylococcus spp.
Teal	E.coli, Salmonella spp.	Staphylococcus aureus
Mallard	E.coli, Salmonella spp.	Staphylococcus aureus
Shoveller	E.coli, Salmonella spp.	Staphylococcus aureus

Table 6: Inhibition Zones of Different Bacteria Against Different Antibiotics

Gadwall	E.coli, Pseudomonas spp.	Staphylococcus aureus
Pouchard	E.coli	Staphylococcus penumonia
Teal	E.coli	Staphylococcus penumonia
Mallard	E.coli	Staphylococcus
Shoveller	E.coli	Staphylococcus

The distribution of several bacterial species among the five sampling sites is depicted in the heat map (Figure 3). Darker hues indicate higher percentages of bacteria, and the color gradient shows the number of bacteria present. This graphic illustrates how the presence of bacteria varies in various places.



Figure 3: Heat Map for Bacterial Distribution at Each Sampling Site, Showing the Prevalence Frequency of Different Bacterial Genera

A total of 16 antimicrobial discs were used and the results were recorded (Table 6).

Bacterial Species	Cip	Nor	Lom	Tob	Cef	Ste	Gen	Tri	Tet	Dox	Ery	Amp	Enr	Azi	Ofl	Сер
E. coli.	R	R	R	S	R	I	R	R	S	R	S	R	I	S	R	S
Klebsiella pneumoniae	R	S	R	S	R	I	S	R	S	R	I	S	R	S	R	S
Enterococcus	R	R	R	S	R	I	1	S	R		S	R	S	R		S
Salmonella ssp.	R	R	R	S	R	I	S	R	S	R	R	R	I	R	R	S
Staphylococcus aureus	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Pseudomonas arignosa	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	
Clostridium septicum	I	R	R	I	R	R	R	R	R	S	S	R	R	R	R	R

The antibiotic susceptibility of different bacterial species is shown in the heat map, where a colour gradient indicates the susceptibility levels (blue for high susceptibility, red for low susceptibility). Patterns of antibiotic efficaciousness and bacterial resistance are highlighted by hierarchical clustering. As an illustration, Clostridium septicum exhibits low susceptibility to doxycycline (Dox) and high susceptibility to ciprofloxacin (Cip). Salmonella species have significant resistance to Amoxicillin(Amp) and Azithromycin(Azi). The diverse ways that bacteria react to different antibiotics are well-illustrated(Figure 4).



Figure 4: Inhibition Zones of Different Bacteria Against Different Antibiotics

DISCUSSION

The River Ravi basin in Pakistan is a significant stopover for many migratory waterfowl species, such as gadwall, common Pouchard, common teal, mallard, and northern Shoveler. These avian species migrate to the area from their breeding habitats in Central Asia and Siberia, in search of milder climates and adequate food supplies over the winter season. [19]. Although migratory wild birds aren't directly linked with the emergence of resistance to antibiotics due to their lack of exposure to antimicrobial drugs, they can act as reservoirs, facilitating the mixing and dissemination of antimicrobial resistance. They also function as a significant measure for assessing the influence of human actions, such as the inappropriate utilization of antimicrobials, on the ecosystem [20-22]. At the first sampling location, Enterococcus had the largest occurrence rate among Gram Positive bacteria, accounting for 41.7%, whereas Pseudomonas was the most prevalent Gram Negative bacterium, with a prevalence of 46.2%. The significance of these results was confirmed by the ANOVA analysis, which showed a significant change (p < 0.05) in the prevalence rates of various bacterial species. Animal and human intestines frequently contain Enterococcus species, and their presence in the environment may be a sign of fecal contamination. The adaptability and capacity of Pseudomonas species to thrive in a variety of settings, including contaminated locations, is well documented. Their high frequency could indicate the existence of contaminants or particular environmental factors that support their proliferation. [23, 24]. In the following sample location, Enterococcus made up 70% of Gram-Positive bacteria, whereas E. coli was present in 54.5%. Significant differences across bacterial species were confirmed by statistical analysis (p < 0.05), highlighting the significance of keeping an eye on the sources of contamination. Location, Gram-positive Enterococcus bacteria represented half the sample specimens, while Gram Negative E. colibacteria made up 47.1%. The elevated E. coli and Enterococcus levels at this location indicate fecal contamination. This consistent observation from numerous areas highlights the broad problem of water contamination in the River Ravi. With a significant P value (p < 0.05), the fourth sample location showed the highest number of Gram-positive bacteria, namely S. aureus, at 45.5%. E. coli was present in 54.5% of Gram Negative bacteria. S. aureus is ubiquitous on human skin and mucous membranes, so its presence in this sample raises concerns about possible contamination caused by people [25]. The theme of fecal contamination is highlighted by the persistence of *E. coli* [26]. In the fifth place where samples were taken, Gram Positive bacteria had a predominance of 26.7% S. aureus and Gram Negative bacteria 55.6% E. coli, with both species showing significant differences in prevalence (p < 0.05). The findings from the fifth site corroborate those from the fourth site, suggesting that human-caused contamination and the presence of feces are ongoing issues.

CONCLUSIONS

It was concluded that the migratory birds contain many Gram-Positive and Gram-Negative pathogenic bacteria. These pathogens have antimicrobial resistance. It's troubling that many infectious bacteria like *Salmonella spp., E. coli*, and *S. aureus* have been isolated from samples. The findings also suggest the fact that many medications have distinct effects on different types of bacteria opens up potential pathways for guided antimicrobial treatment, in addition to the required constant monitoring and individualized treatment plans. Protecting human and animal health necessitates better management and monitoring of environmental contamination with bird feces. The migratory birds could play a vital role in the dispersal of AMR bacteria.

Authors Contribution

Conceptualization: BNK Methodology: BNK, ST Formal analysis: BNK, YA Writing-review and editing: BNK, ST, YA, AM, MAH, MF, SG, AT 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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