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Bugs and Drugs: A Three-Year Susceptibility Pattern of Lower Respiratory Tract Bacterial Pathogens in Karachi

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ABSTRACT

OBJECTIVE: To ascertain the bacteriological profile of respiratory tract infections within the local population of Karachi, including antibiotic sensitivity and resistance patterns.

METHODOLOGY: This Retrospective laboratory-based surveillance study was conducted at the Department of Microbiology Section, Dow Diagnostic Research and Reference Laboratory (DDRRL) from 1st January 2021 to 31st December 2023. Data were collected from medical records for the sputum samples registered at the microbiology section of DDRRL for culture and sensitivity tests.

RESULTS: A total of 12187 sputum samples were collected, of which 6013 (49.3%) were positive, with 3452 (57.4%) male and 2561 (42.6%) female. *Moraxella catarrhalis* was the most frequently isolated microorganism (2664, 44%) followed by *Pseudomonas aeruginosa* (1640, 27%) and *Haemophilus* species (459, 8%). Among Gram-negative bacteria, *Pseudomonas aeruginosa* and *Haemophilus* species were sensitive to most of the antibiotics. In contrast, *Acinetobacter species*, *E. coli* and *Enterobacter species* showed high resistance to the majority of antibiotics. Minocycline and Tigecycline appeared highly sensitive, whereas Cotrimoxazole and Levofloxacin appeared resistant to most Gram-negative bacteria. Among Gram-positive bacteria, Streptococcus pneumoniae showed strong resistance to Cotrimoxazole, and Staphylococcus aureus showed strong resistance to Erythromycin. For Gram-positive bacteria, Linezolid, Chloramphenicol and Vancomycin were highly sensitive antibiotics. Overall, Cotrimoxazole appeared resistant, and Chloramphenicol appeared sensitive in both Gram-positive and Gram-negative groups.

CONCLUSION: *Moraxella catarrhalis, Pseudomonas aeruginosa* and *Haemophilus species* were the most isolated pathogens. They were sensitive to Minocycline and Tetracycline while resistant to Cotrimoxazole and Levofloxacin.

KEYWORDS: Sputum culture, antimicrobial resistance (AMR), *Pseudomonas aeruginosa. Streptococcus pneumoniae*, Antibiotic susceptibility testing (AST), Respiratory tract infections

INTRODUCTION

Lower respiratory tract infections (LRTIs) are a widespread and varied category of infections that affect both hospitalized and non-hospitalized patients. Pneumonia, bronchitis, bronchiolitis, abscess formation, emphysema, and tuberculosis are all examples of LRTIs[1]. LRTIs are responsible for morbidity and mortality, with increased medical expenses. They are responsible for 4.4% of all hospital admissions and 2.74 million deaths per year worldwide[2]. The incidence and fatality rates of LRTI fluctuate based on age, gender, season, at-risk population characteristics, distribution of causative agents, prevalence of antimicrobial resistance, and primarily patterns of drug susceptibility. Risk factors such as smoking, chronic obstructive pulmonary disease, structural pulmonary disease, diabetes mellitus, altered mental status, and chronic alcoholism exacerbate the onset and advancement of the disease. *Pseudomonas aeruginosa (P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*) are bacteria commonly found in people with LRTI. P. aeruginosa is more common in adult respiratory wards and intensive care units, while S. aureus is more common in paediatric wards[3]. Acinetobacter baumannii and Klebsiella pneumoniae are also common, especially in intensive care units, and they are linked to increased levels of antibiotic resistance[3,4]. People who get pneumonia from the community often have *Haemophilus influenzae (H. influenza)* and *Streptococcus pneumoniae (S. pneumonia)* regularly at the same frequency. *H. influenzae* makes some β-lactamase, but *S. pneumoniae* doesn't seem to be very resistant to penicillin[5].
Streptococcus pneumoniae, Klebsiella pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Pseudomonas aeruginosa, Citrobacter, Escherichia coli, Enterococcus spp., and *Acinetobacter baumannii* are the most prevalent bacterial infections in LRTIs worldwide[6,7]. Up to 10% of patients receiving invasive mechanical ventilation and approximately 1% of patients overall may develop hospital-acquired pneumonia (HAP)[8]. HAP is more likely to be multidrug-resistant because hospitals lack sufficient isolation facilities, especially in developing countries. This makes treatment less effective. The death rates for ventilator-associated pneumonia and hospital-acquired pneumonia can be as high as 50% and 20%, respectively, depending on the person's other health problems[9]. Multidrug-resistant strains pose a threat to healthcare worldwide. The emergence of pan-resistant bacteria is thought to make many infections impossible to treat. Multidrug resistance (MDR) can happen for several reasons. The molecular basis of this phenomenon involves the transfer of resistance genes to bacteria via transformation, conjugation, transduction, and other mechanisms. The antibiotic efflux system, bacterial enzymatic drug modifications, and genetic changes in drug targets are additional ways that drugs can become resistant. Other factors that lead to the development of resistant bacteria include incorrect use of antimicrobial agents, spread of resistant bacteria among patients and health care personnel, and inadequate guidance on their use. Using broad-spectrum antibiotics too often is also making things worse[10].
Despite the global data available on LRTIs and antimicrobial resistance, there is a lack of large-scale, local surveillance data from Karachi that reflects both community-acquired and hospital-acquired infections. The growing burden of multidrug-resistant pathogens in respiratory samples poses serious challenges for empirical treatment and antibiotic stewardship. Hence, a localized understanding of pathogen prevalence and resistance trends is essential to guide evidence-based clinical decisions and update treatment protocols in this setting. The rationale for conducting this study was to ascertain patterns of antibiotic sensitivity and resistance, along with the bacteriological profile of respiratory tract infections within the Karachi local population.

METHODOLOGY

This retrospective laboratory-based surveillance study was conducted at the Microbiology section of Dow Diagnostic Research and Reference Laboratory (DDRRL) from 1st January 2021 to 31st December 2023. All qualifying lower respiratory tract samples collected for culture and sensitivity testing throughout the study period were included in the analysis, regardless of any predetermined minimum sample size. Culture and sensitivity reports of sputum were retrieved from the medical records of the Microbiology section of DDRRL through the Laboratory Information System software, after obtaining Institutional Review Board (IRB) approval, with Reference No. IRB-3270/DUHS/EXEMPTION/2023/479. Samples with incomplete information or with insignificant / no bacterial growth were excluded from the study. All samples were processed as per CLSI guidelines 2021-23 for standard microbiological procedures and antibiotic susceptibility testing, and defined clinical breakpoints were used (11). The culture reports included samples from both hospitalized and non-hospitalized patients. The antibiotic discs tested for susceptibility included Amikacin (30µg), Ampicillin (10µg), Amoxicillin / Clavulanic acid (30µg), Aztreonam (30µg),), Cefoxitin (30µg), Ceftriaxone (30µg), Cefixime (5µg), Cefuroxime (30µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Ceftazidime avibactam (30/20µg), Cotrimoxazole (25µg), Chloramphenicol (30µg), Clindamycin (2µg), Erythromycin (15µg), Fusidic acid (10µg), Gentamicin (10µg), Levofloxacin(5µg), Linezolid (30µg), Meropenem (10µg), Minocycline (30µg), Moxifloxacin (5µg), Piperacillin Tazobactam (100/10µg), Tobramycin (10µg), Tetracycline (30ug), Tigecycline (15µg), Penicillin (10 units) and Vancomycin (30µg). Nevertheless, MICs of Colistin (2 and 4 µg/mL) were tested by the Colistin agar dilution method according to the CLSI protocol.

Frequencies and percentages were reported using descriptive statistics using SPSS version 16. Cross-tabulation was used for statistical analysis to assess the prevalence of isolated pathogens across age groups and the sensitivity patterns of these pathogens to different antibiotics.

RESULTS

The total number of sputum samples submitted for culture and sensitivity testing during the study period, from 1st January 2021 to 31st December 2023, was 12187, of which 6013 (49.3%) were positive, with 3452 (57.4%) male and 2561 (42.6%) female. Most of the patients were in age range of 50 to 60 years (1220, 20.5%) with mean age of 50 years. *Moraxella catarrhalis* was the most isolated microorganism (n=2664, 44%) followed by *Pseudomonas aeruginosa* (1640, 27%). Frequencies and percentages of different microorganisms isolated from sputum samples are given in **Table I** and age wise distribution is presented in **Figure 1**. Among Gram negative bacteria, *Pseudomonas aeruginosa* and *Haemophilus species* were sensitive to most of the drugs while *Acinetobacter species*, *E. coli* and *Enterobacter species* showed highly resistant to majority of the antibiotics. Minocycline and Tigecycline appeared as highly sensitive whereas Cotrimoxazole and Levofloxacin seem to be resistant in majority of gram negatives. Colistin revealed intermediate sensitivity in most of the gram-negative bacteria. Taking into consideration the gram-positive bacteria, *Streptococcus pneumonia* showed high resistance to Cotrimoxazole, while *Staphylococcus aureus* was highly resistant to Erythromycin (70%) and Methicillin-resistant *Staphylococcus aureus* (MRSA*)* appeared as 63%. Linezolid and Chloramphenicol were found to be highly sensitive antibiotics for gram positive bacteria. Overall, Cotrimoxazole appeared resistant and Chloramphenicol as sensitive in both gram positive and negative bacteria. Antibiotic Profile (Percent Resistance) against isolated organisms from sputum are given in **Table II**.

Table I: Frequencies with percentages of Organisms isolated from sputum culture and sensitivity

Organisms	Frequency (%)
Moraxella catarrhalis	2664 (44)
Pseudomonas aeruginosa	1640 (27)
Haemophilus species	459 (8)
Klebsiella pneumoniae	351 (6)
Acinetobacter species	312 (5)
Staphylococcus aureus	228 (4)
Escherichia coli	151 (2.5)
Streptococcus pneumoniae	149 (2.5)
Enterobacter species	32 (0.5)
Burkholderia cepacia	9 (0.15)
Nocardia species	4 (0.06)
Stenotrophomonas maltophilia	4 (0.06)
Aeromonas	3 (0.05)
Serratia species	2 (0.03)
Elizabethkingia meningoseptica	1 (0.015)
Flavobacterium species	1(0.015)
Proteus mirabilis	1 (0.015)
Providencia species	1 (0.015)
Salmonella typhi	1 (0.015)

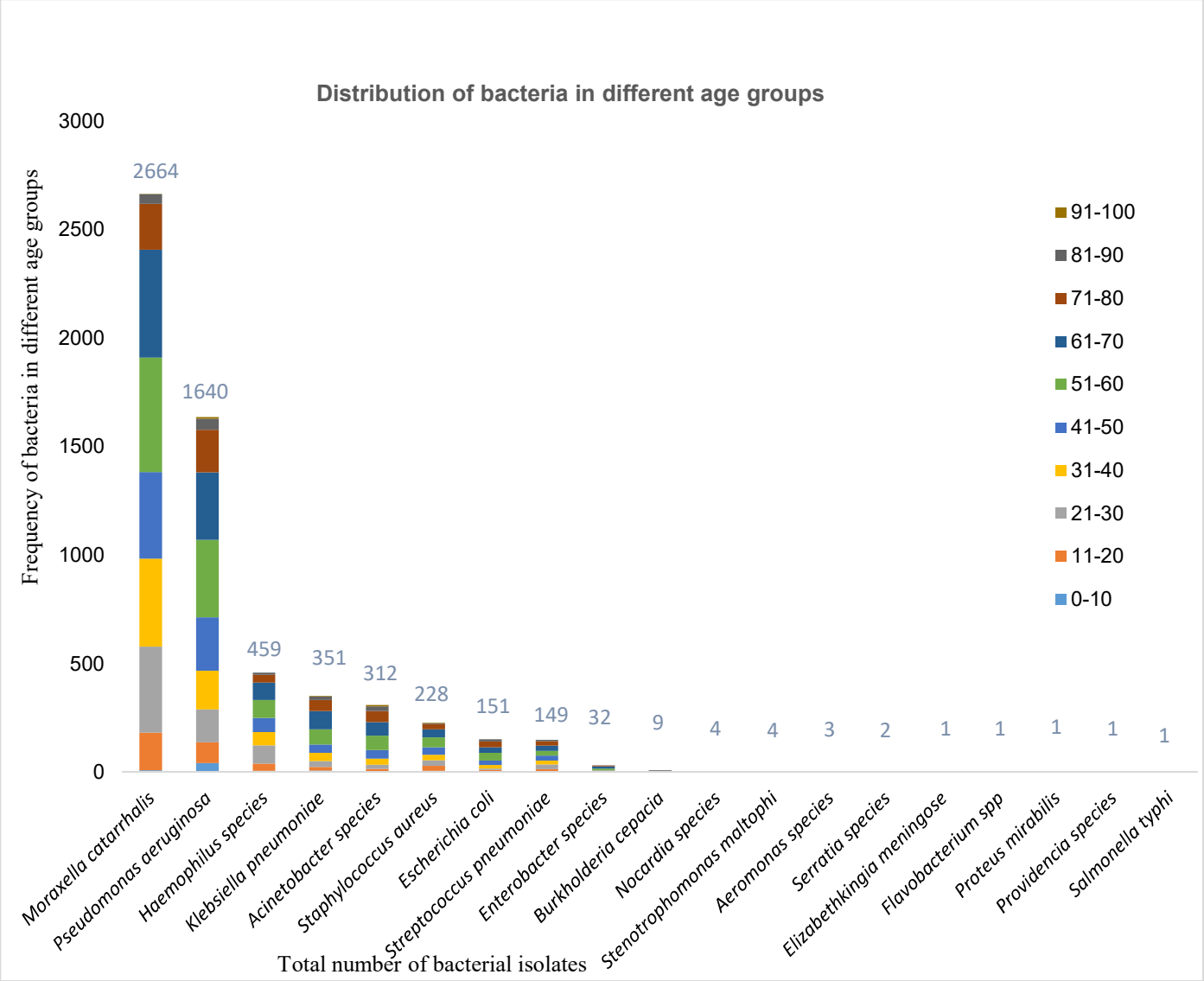

Figure 1: Frequency Distribution of Bacteria in different age groups

Table II: Antibiotic Profile (Percent Resistance) against isolated organisms from sputum

Organisms names n=6013 (100%)	Gram Negative							Gram positive	
	Moraxella catarhalis n=2664 (44%)	*Pseudomonas aeruginosa n=1640 (27%)*	*Hemophilus species n=459 (8%)*	*Klebsiella pneumonia n=351 (6%)*	*Acinetobacter species n=312 (5%)*	*Escherichia coli n=151 (2.5%)*	*Enterobacter species n= 32 (0.5%)*	*Staphylococcus aureus n=228 (4%)*	*Streptococcus pneumoniae n=149 (2.5%)*
Penicillins									
P	NT	NT	NT	NT	NT	NT	NT	NT	6
FOX	NT	NT	NT	NT	NT	NT	NT	63	NT
AMP	NT	NT	6	NT	NT	94	NT	NT	6
Beta lactam Combination agent									
AMC	1	NT	0	49	NT	67	NT	63	NT
TZP	NT	5	NT	27	86	41	56	NT	NT
SCF	NT	NT	NT	32	58	32	67	NT	NT
CZA	NT	3	NT	16	95	11	NT	NT	NT
Cephalosporins									
CFM	33	NT	5	61	NT	87	90	NT	NT
CXM	NT	NT	NT	63	NT	89	87	NT	NT
CAZ	NT	6	NT	NT	87	NT	NT	NT	NT
CRO	1	NT	0	54	95	83	83	NT	5
Monobactams									
ATM	NT	7	NT	38	NT	67	67	NT	NT
Carbapenem									
MEM	NT	23	NT	32	88	26	68	NT	NT
Glycopeptide									
VA	NT	NT	NT	NT	NT	NT	NT	0	0
Lipopeptides									
CT	NT	4	NT	6	3	0	2	NT	NT
Aminoglycosides									
CN	NT	9	NT	29	79	27	55	22	NT
AK	NT	NT	NT	27	80	14	56	NT	NT
TOB	NT	6	NT	32	76	23	44	NT	NT
Tetracycline									
TE	32	NT	NT	NT	NT	NT	NT	39	25
MH	NT	NT	NT	19	3	12	35	NT	NT
TGC	NT	NT	NT	11	NT	6	11	NT	NT
Quinolones									
CIP	NT	10	NT	40	86	82	68	NT	NT
LEV	NT	11	NT	55	87	72	90	NT	15
MXF	75	NT	39	NT	NT	NT	NT	NT	NT
Folate pathway antagonist									
SXT	92	NT	73	52	75	70	61	31	83
Phenicols									
C	1	NT	0	NT	NT	NT	NT	7	6
Macrolides									
E	74	NT	NT	NT	NT	NT	NT	70	43
CLR	NT	NT	44	NT	NT	NT	NT	NT	NT
Lincosamides									
DA	NT	NT	NT	NT	NT	NT	NT	41	27
Oxazolidinones									
LZD	NT	NT	NT	NT	NT	NT	NT	0	0
Steroid									
FD	NT	NT	NT	NT	NT	NT	NT	28	NT

>80% Resistance, <20% Resistance, Not Tested

P:Penicillin, FOX:Cefoxitin, AMP:Ampicillin, AMC:Amoxicillin/Clavulanic acid, TZP:PipracillinTazobactam,SCF:cefoperazone/sulbactam, CZA:Ceftazidime avibactam, CFM: Cefixime, CXM:Cefuroxime, CAZ: Ceftazidime, CRO:Certriaxone, ATM:Aztreonam, MEM:Meropenem, VA:Vancomycin, CT:Colistin, CN:Gentamicin, AK:Amikacin, TOB:Tobramycin, TE:Tetracycline,MH:Minocycline, TGC:Tigecycline, CIP:Ciprofloxacin, LEV:Levofloxacin, MXF:Moxifloxacin, SXT:Cotrimoxazole, C:Chloramphenicol, E:Erythromycin, CLR:Clarithromycin, DA:Clindamycin, LZD:Linezolid, FD:Fusidic acid

DISCUSSION

In our investigation, most of the patients with lower respiratory tract infections (LRTIs) were men between the ages of 50 and 70. This aligns with both national and international data that underscore heightened vulnerability in older persons, especially males, attributable to immunosenescence, a greater incidence of chronic illnesses, and lifestyle-related risk factors such as smoking[12-14]. Respiratory diseases are more common in children worldwide, but they are mostly viral rather than bacterial[15]. *Moraxella catarrhalis* (44%) was the most frequently detected microbe in our investigation. *Pseudomonas aeruginosa* (27%) and *Haemophilus species* (8%) came next. Regional studies from China and Iran have revealed similar outcomes[16,17]. *Staphylococcus aureus* (4%) and *Streptococcus pneumoniae* (2.5%) were the most common Gram-positive organisms. This is in line with data from Lahore, Pakistan[18], and international research such as Santella B et al. [19].

In terms of antimicrobial susceptibility, *M. catarrhalis* was highly sensitive to chloramphenicol, ceftriaxone, and amoxicillin-clavulanic acid, but resistant to cotrimoxazole, erythromycin, and moxifloxacin. These results align with the findings of Dao DT et al. [20], who documented comparable susceptibility patterns.

According to Valzano F et al.[21], *Pseudomonas aeruginosa* was sensitive to the majority of medications, including Aztreonam, Ceftazidime-avibactam, Colistin, Gentamicin, Piperacillin/tazobactam, and Ceftazidime; nevertheless, Nasrin S et al.[22] also observed 23% resistance to Meropenem. *Haemophilus species* were sensitive to the majority of antibiotics, except Co-trimoxazole, findings also reported by Kiedrowska M et al.23, but not supported by Qin Wang C-GL et al.[24], who mentioned Ampicillin resistance as the highest, followed by Cefaclor and Trimethoprim-sulfamethoxazole. *Acinetobacter species* were the most resistant among Gram-negative bacteria, with more than 85% resistance to several antibiotics, including cephalosporins such as ceftriaxone, carbapenems such as meropenem, and aminoglycosides. Only colistin (97%), minocycline (97%), and Tigecycline (92%) showed high sensitivity, which is in line with Bilal H et al. [25] and others[19,26]. However, emerging resistance to colistin has been increasingly reported, raising significant alarm[27]. *Enterobacter* and *E. coli* species were also quite resistant to third-generation cephalosporins and fluoroquinolones, but they remained sensitive to Tigecycline, colistin, and minocycline[27,28].

S. aureus was resistant to erythromycin (70%) and cefoxitin (63%) among Gram-positive organisms, although susceptible to vancomycin, linezolid, and chloramphenicol. These results align with regional research, including that of Santella B et al.19, who reported that *S. pneumoniae* exhibited resistance to cotrimoxazole, erythromycin, and clindamycin, but remained sensitive to amoxicillin-clavulanic acid, ceftriaxone, and vancomycin[30].

The fact that *Acinetobacter* and *Enterobacterales* have high resistance rates underscores the growing problem of multidrug-resistant organisms (MDROs), especially for patients who are very sick or have weak immune systems. The continued effectiveness of reserve medications such as colistin and Tigecycline underscores the necessity of maintaining their utilization through robust antimicrobial stewardship initiatives. These findings highlight the pressing necessity for localized, evidence-based empirical therapy guidelines.

It is also necessary to monitor antimicrobial resistance (AMR) in the bacteria abundant in the area. This will help with infection control and stop the spread of resistant strains. The WHO's Global Action Plan on AMR states that organizations should regularly update and analyze their antibiogram data, adjust their infection control practices, and prescribe antibiotics only when necessary.

This study is hampered by its retrospective methodology and the absence of clinical association with patient outcomes; however, the substantial sample size and the inclusion of both outpatient and inpatient populations augment the reliability and applicability of the findings. These observations provide a significant foundation for forthcoming surveillance and policy formulation in Karachi and analogous urban healthcare environments; however, trend-based analyses are recommended for the future.

CONCLUSION

This study underscores that Gram-negative bacteria, including *Moraxella catarrhalis, Pseudomonas aeruginosa,* and *Haemophilus species*, were the predominant pathogens isolated in lower respiratory tract infections (LRTIs) within the local population. These organisms showed a strong response to minocycline and tetracycline, but not to cotrimoxazole and levofloxacin. A considerable number of *Staphylococcus aureus* strains among Gram-positive isolates were classified as *MRSA,* exhibiting resistance to erythromycin while remaining susceptible to vancomycin, chloramphenicol, and linezolid. *Streptococcus pneumoniae* exhibited resistance to cotrimoxazole while remaining susceptible to ampicillin, amoxicillin-clavulanic acid, ceftriaxone, and levofloxacin.

In general, cotrimoxazole didn't work very well on either type of bacteria. Chloramphenicol demonstrated promising in vitro efficacy against specific respiratory infections; however, its clinical use is constrained by the potential for significant hematological toxicity. Consequently, its prospective role must be assessed with prudence and considered solely in specific clinical situations where safer alternatives are not available - warranting further evaluation before being considered for clinical use.

Ethical Permission: Dow University of Health Sciences, Karachi, Pakistan, IRB exemption approval letter No. IRB-3270/DUHS/EXEMPTION/2023/479.
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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Naseem S: Critical revision and interpretation of data
Arshad F: Manuscript writing and final approval
Fatima A: Study conception, design and final approval
Zehra A: Statistical analysis
Naz A: Interpretation of data and literature search
Siddiqui S: Literature search, acquisition of data and data analysis

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