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ANTIBIOTIC RESISTANCE PATTERNS IN RESPIRATORY TRACT INFECTIONS IN SINDH

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Abstract

Background: *Respiratory Tract Infection (RTI) present a major global health challenge, being the lethal communicable diseases and the third most common cause of death worldwide, after ischemic heart disease and cerebrovascular diseases. The growing issue of antibiotic resistance is an international public health crisis, primarily caused by the over-prescription of antibiotics.*

Methodology: *The research was conducted at the Medical Research Centre and data was collected from Diagnostic and Research (DR) Laboratory of LUMHS, located in Jamshoro, Pakistan. The data regarding infections were retrieved from the laboratory records of sputum cultures performed over past six months.*

Result: *A total of 600 sputum culture data was retrieved, out of which, 343 (57.2%) sputum samples were isolation-positive from males, while 257 (42.8%) were isolation-positive from females. The primary causative organism of RTIs was Pseudomonas aeruginosa, with 253 positive cases (42.2% total). This was followed by Klebsiella pneumoniae, with 243 cases (40.5%). Antibiotic susceptibility patterns showed Meropenem (85% sensitive, 15% resistant), Piperacillin/Tazobactam (84% sensitive, 16% resistant) Amikacin (82% sensitive, 18% resistant), and Colistin (100% sensitive), while Ceftazidime (71% sensitive, 29% resistant) and Ciprofloxacin (50% sensitive, 50% resistant) were moderately effective. Less effective antibiotics included Aztreonam (54% sensitive, 46% resistant), Ceftriaxone (28% sensitive, 72% resistant), Cefixime (22% sensitive, 78% resistant), and Ampicillin (0.4% sensitive, 99.6% resistant).*

Conclusion: *Antibiotics resistance to RTI in Sindh was observed, with a striking predominance of Pseudomonas aeruginosa and Klebsiella pneumoniae, particularly affecting elderly males. Colistin, Meropenem, and Piperacillin/Tazobactam were effective; however, first-line antibiotics such as Ampicillin and Cefixime exhibited high resistance in this study. The study*

emphasizes the importance of culture-based prescribing and highlights the need for improved diagnostic capacity and AMR surveillance on a regional scale. Public education regarding antibiotic misuse was implemented, and future research in the areas of the rising threat of multidrug resistance and the opposing goal of achieving effective treatment outcomes is recommended.

INTRODUCTION

Antibiotic resistance among respiratory pathogens is aggregate emergently. Respiratory Tract Infection (RTI) present a major global health challenge, being the lethal communicable diseases and the third most common cause of death worldwide, after ischemic heart disease and cerebrovascular diseases. RTI are the most common infections of human being (1, 2). There are two different types of RTIs: lower RTIs and upper RTIs (3).Lower RTIs are among the frequently reported infectious diseases of human in worldwide (3). The causative agents may include fungi, viruses, or bacteria. Streptococcus pneumoniae, Ps. aeruginosa, K. pneumoniae, and Staphylococcus aureus are among the prominent bacteria that cause lower respiratory tract infections (4). About 10% of the worldwide morbidity and mortality is caused by RTI by microbes and sometimes due to viral infection. Streptococcus pneumonia is the most common pathogen in RTI.

Bacterial etiology may differ in diverse geographical regions and even over time in the similar location and population, routine surveillance of microbial etiology of LRTI is significant (5). In Southeast Asia, RTI is responsible for over one-third of reported mortality (3). Several factors can influence the incidence and mortality associated with RTI. These factors include the distribution of causative agents, the size of the at-risk population, the season, age, gender, the incidence of antibiotic resistance, and particular characteristics of the at-risk population. In primary health care settings, RTIs are a typical reason for consultations, and antibiotics are frequently prescribed. RTIs account for approximately 60% of all antibiotic prescriptions in primary care (6).The rising morbidity and mortality from these infections is a growing concern for healthcare practitioners.

The choice of antimicrobial therapy for bacterial LRTIs is relatively straight forward when the etiologic agents and their antibiotic susceptibility patterns are known. However, the clinical presentation is usually not specific enough to make a firm etiologic diagnosis whether in the community or hospital setting (7). The unhygienic environment, poor management, inexperienced professionals, and misuse or overuse of antibiotics contribute to the alarming rise of antibiotic-resistant bacteria due to the acquisition of resistant genes by bacteria.

A recent research report shows that In Pakistan the respiratory infections are also the common reason to visit a physician in primary care. Majority of the patients consulting a general practitioner with signs and symptoms of respiratory infections are frequently treated empirically (8). The World Health Organization (WHO) estimates that 2 million of the children under five years of age die of pneumonia each year in Pakistan (9). Antibiotic misuse is prevalent in low and middle-income nations, including Pakistan (10) The incorrect use of antibiotics is common globally among children with acute respiratory infections (11).Nowadays, antibiotic resistance exerted by microorganisms against antibiotics is considered as a serious issue by global medicinal and research community. The objective of the study is to evaluate and document the predominant bacterial pathogens and their ABR patterns among patients.

RATIONALE OF STUDY

There is limited literature available evaluating current pattern of antibiotic resistance in RTI in Pakistan. This study addresses a critical gape in identifying the specific infectious agents responsible for respiratory tract infections and assessing the pattern of antibiotic resistance in respiratory tract infections in Sindh that will contribute to the broader understanding of this issue and will help to identify specific resistance trends in the Sindh. This information is vital for public health planning, resource allocation, and the development of targeted intervention strategies to reduce the burden of respiratory infections in the region.

PURPOSE OF STUDY

To evaluate the pattern of antibiotic resistance in Sindh.

Objectives:

- To evaluate infectious organisms in patients presenting with respiratory tract infections in Sindh.
- To evaluate the pattern of antibiotic resistance in these patients.

Materials and Methods

Study Setting: The research was conducted at Medical Research Centre, and data regarding infections were collected from the Diagnostic and Research (DR) Laboratory of LUMHS, located in Jamshoro, Pakistan. This laboratory performs routine microbiological testing, such as blood and sputum cultures, and acts as a reference center for diagnosing bacterial infections in the area.

Study Design: Cross-sectional Study design was used. This approach was selected as it allows for the examination of bacterial strains isolated from sputum cultures and their associated patterns of ABR at a specific moment in time.

Study Period: The data was collected for a period of six months, from July 2024 till December 2024, commencing upon receiving approval from the Ethical Review Committee (ERC) and Advance studies research board (AS&RB) at Liaquat University of Medical and Health Sciences, Jamshoro. Data collection started promptly after receiving the approval and persisted until the conclusion of the specified period.

Sampling Technique: A non-probability Convenient Sampling Technique was employed to select the study sample. This non-probability sampling method was chosen due to its practicality and effectiveness in gathering data from accessible records within the six-month study duration. The DR laboratory supplied retrospective data on all sputum culture tests conducted during the said period.

Sample Size: The sample size was determined from the laboratory records of sputum cultures performed over six months, during which 600 positive sputum culture reports were assessed. A thorough review of all records was conducted to identify suitable cases that met the inclusion criteria.

Inclusion Criteria and Exclusion Criteria: All patients whose sputum culture records were available at the DR laboratory, LUH, Hyderabad, within the study period. Patients of any age, gender, and clinical background who have undergone sputum culture tests were also included. Patients whose sputum culture records were not available in the DR laboratory database and incomplete or missing records for sputum cultures were also excluded.

Data Collection Tool: Data was gathered through a retrospective analysis of laboratory records. The records contained comprehensive information on Patient demographics (ID, age, gender, address), the bacterial species identified in sputum cultures, the antibiotic susceptibility profiles of the identified bacterial strains and information related to the types of infections (e.g., respiratory). Data was retrieved from existing laboratory databases and, where necessary, physical records. A standardized data collection form was structured to extract pertinent information, ensuring both consistency and accuracy.

Statistical Analysis:

Data was imported into SPSS version 22 after being entered into Microsoft Excel for statistical analysis. The following analyses were performed: Questionnaire and laboratory data were collected in Microsoft Excel and statistical analysis was performed using SPSS 22.0 software (IBM, Armonk, NY, USA). Descriptive statistics are presented as frequencies and percentages for categorical variables such as gender, bacterial species, and ABR profiles. Measures of central tendency (mean, median) for continuous variables such as age; the prevalence of ABR for each bacterial strain was calculated and presented as percentages. Distribution of antibiotic classes used and resistance rates for each class. All results were displayed in tables, charts, and graphs to ensure clear and comprehensible data presentation.

Experimental Work: The Kirby-Bauer disc diffusion method was employed with Muller Hinton agar plates to determine the antibiotic sensitivity patterns of isolated microorganisms. Based on the zone of inhibition, the isolates were classified as either susceptible or resistant according to the standards established by the Diagnostic and Research Laboratory.

The surface of Muller Hinton agar plates was streaked by a sterile cotton swab. The Muller Hinton agar plates were allowed to dry before applying antibiotic disc. Then, to allow the antibiotics to diffuse into the agar medium, filter paper disks impregnated with a predetermined quantity of antimicrobial medications were carefully and carefully placed on the agar plates. The plates were then allowed to sit at room temperature for an hour. After that, the plates were incubated for 24 hours at 37°C. Antimicrobial activity on the plates was shown by an inhibitory zone that developed around the disc. The antibiotics tested were Penicillin's, cephalosporins, Meropenem, Amikacin, Aztreonam, Piperacillin, Ciprofloxacin, Ceftriaxone and Cefixime. Type of organisms most common in the sputum sample were noted and drugs still effective for the particular organism were noted.

Ethical Considerations: Ethical approval of the study protocol was obtained from the Ethical Review Committee (ERC) of LUMHS, Jamshoro and Advance studies research board (AS&RB) at Liaquat University of Medical and Health Sciences, Jamshoro before the conduct of the study. As the study involves retrospective data collection from existing laboratory records, patient consent was not required. All patient data was handled with the extreme confidentiality. Each patient was given a **study code number** to safeguard their identity. Only de-identified data was utilized in the study, and it was assured to participants that their personal information would not be disclosed or published. The data was stored in password protected files, available only to authorized personnel. Upon completion of the study, all physical and digital records were archived for potential future reference, in accordance with the university's data retention policies.

RESULTS

Table 01. Socio-demographic profile of the study subjects N=600

Variable	Frequency	Percentage
Gender Distribution		
Male	343	57.2
Female	257	42.8
Age Distribution		
15-30 Years	129	21.5
31-45 Years	113	18.8
46-60 Years	144	24.0
61-100 Years	214	35.7

Above table shows that out of 600 subject, 343 (57.2%) sputum samples were isolation-positive from male patients, while 257 (42.8%) were isolation-positive from female patients, showing a definite male predominance in RTI patients. This trend suggests that respiratory infections cause more damage in males than in females. Moreover, Age distribution shows out of 600 patients RTI 214 (35.7%)belonged to 61 to 100 years of age, followed by 144 (24%) between 46–60 age group, then 129 (21.5%) were between 15 to 30 years, and then113 (18.8%) for the 31 to 45 years' age group.

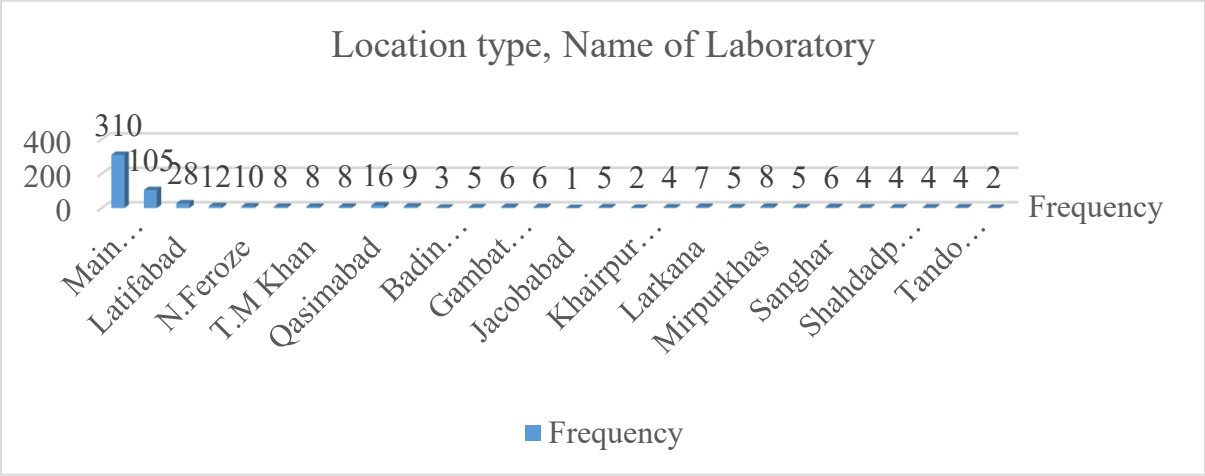


Figure 01. RTI Patient distribution according to laboratory location

The distribution of patients by location shows that the majority of cases 310 came from the main branch of DR Lab, LUH, Hyderabad. Additionally, there were 105 patients from the Jamshoro LUMHS branch, 28 from Latifabad, Hyderabad and 16 from the Qasimabad, Hyderabad laboratory. The remaining cases were reported from branches across Sindh as presented in Figure 1.

TABLE 02. Growth of the Organism in the Sputum Sample

Name Of Identified Organism	Frequency	Percentage	Cumulative percentage
PS. Aeruginosa	253	42.2%	42.2%
Klebsiella Pneumoniae	243	40.5%	82.7%
Pseudomonas species	50	8.3%	91%
others (*Proteus mirabilis Acinetobacter species Escherichia coli*)	54	9.0%	100%

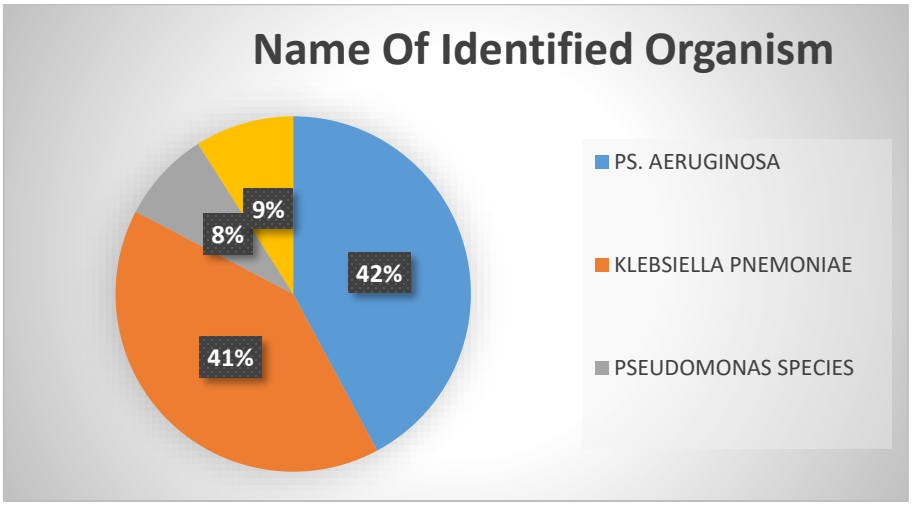


Figure .2, presenting Growth of the Organism in the Sputum Sample. The most dominant and prevalent causative organism of RTI was identified as Pseudomonas aeruginosa, with 253 positive cases, accounting for 42.2% of the total. This was followed by Klebsiella pneumoniae, which had 243 cases (40.5%). Other organisms

included various Pseudomonas species (8.3%), Proteus mirabilis, Acinetobacter species, and Escherichia coli, each contributing to the remaining cases (8.3%).

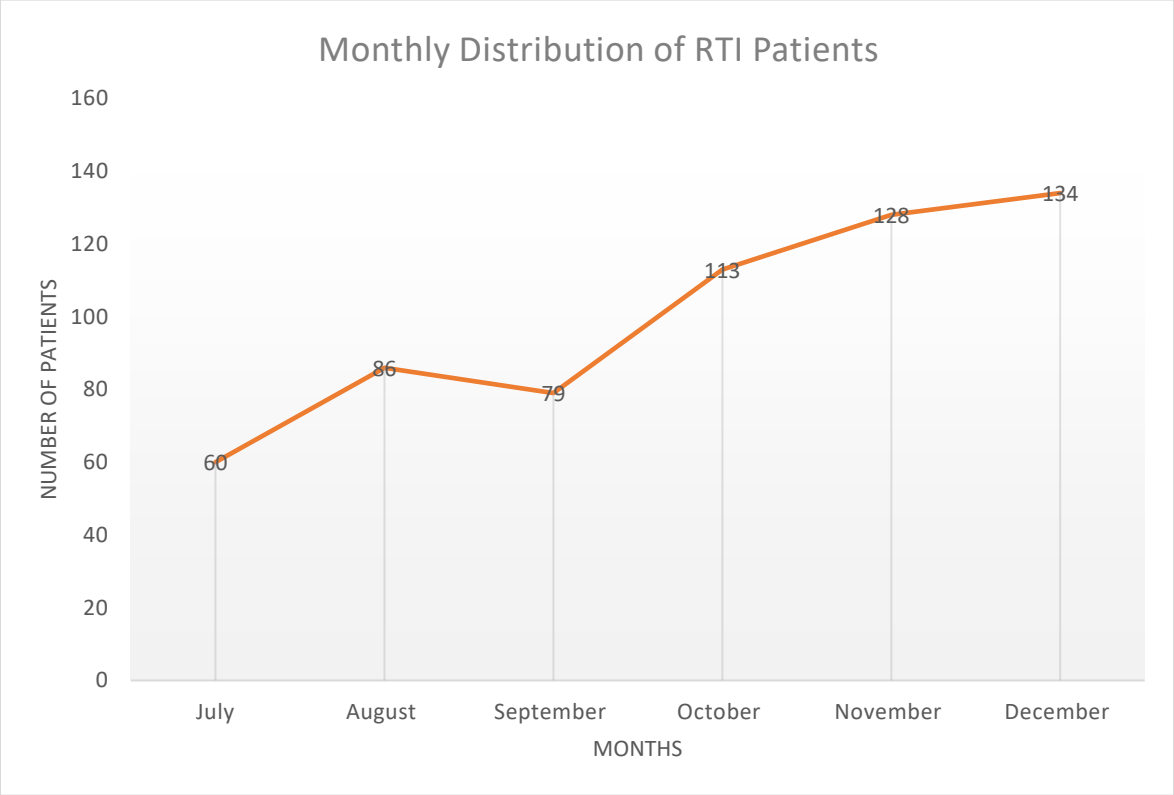


Figure.1 shows the Monthly Distribution of Patients with RTI

Above figure presenting the monthly distribution of patients with respiratory tract infections. The recording of variation in RTI patients per the calendar month indicated only a low variation. In October, November, and December, the number of cases increased steadily and then came back to the level equivalent to all other months

Table .3 Antibiotics susceptibility and resistant pattern of overall microorganisms

Antibiotics	No. of Sensitive isolates (%)	No. of isolates Resistant isolates (%)	No. of isolates tested
Meropenem	418 (85%)	76 (15%)	494
Amikacin	406(82%)	88 (18%)	494
Aztreonam	267 (54%)	228 (46%)	495
Piperacillin/tazobactam	414 (84%)	77 (16%)	491
Ceftazidime	175 (71%)	72 (29%)	247
Ciprofloxacin	248 (50%)	247 (50%)	495
Colistin	58 (100%)	00 (0%)	58
Ceftriaxone	69(28%)	180 (72%)	249
Cefixime	55 (22%)	191 (78%)	246
Ampicillin	1 (0.003%)	251(99.6%)	252

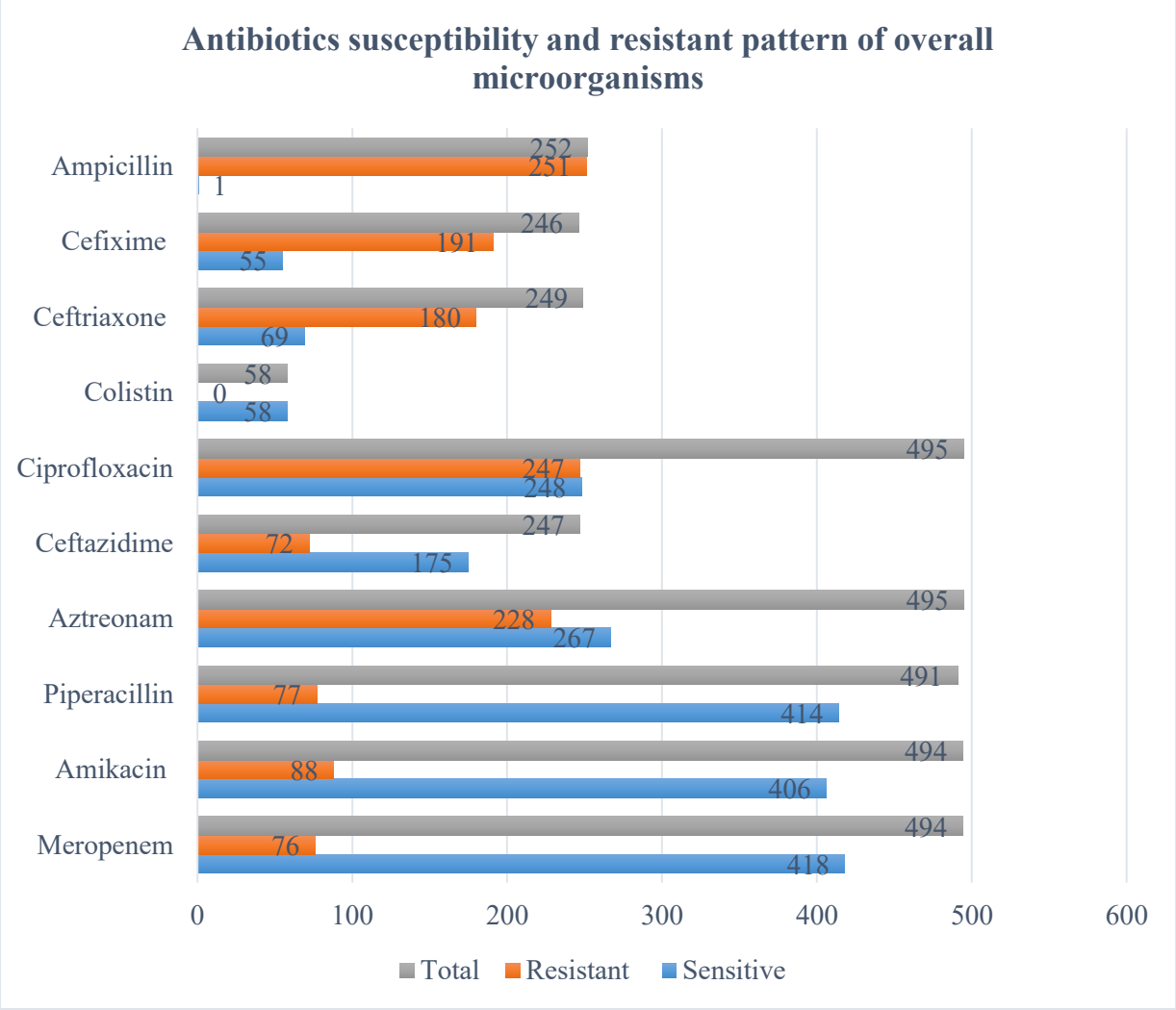


Figure 2. Antibiotics susceptibility and resistant pattern of overall microorganisms

The table 3. Describing antimicrobial susceptibility and resistance patterns of various antibiotics against microorganisms, Meropenem (85% sensitive, 15% resistant), Amikacin (82% sensitive, 18% resistant), Piperacillin/Tazobactam (84% sensitive, 16% resistant), and Colistin (100% sensitive, 0% resistance. Moderately Effective Antibiotics include Ceftazidime (71% sensitivity, 29% resistance) and Ciprofloxacin (50% sensitivity, 50% resistance), which has a high resistance rate, making it less reliable for treatment. Less effective antibiotics include Aztreonam (54% sensitive, 46% resistant), Ceftriaxone (28% sensitive, 72% resistant), Cefixime (22% sensitive, 78% resistant), and Ampicillin (0.4% sensitive, 99.6% resistant). Nearly all tested isolates show resistance, making these antibiotics almost completely ineffective

Table .5. Relationship between the Patient's Age and the Isolated Organism Type

Age	Organisms isolated				Total
	Ps. Aeruginosa	Klebsiella Pneumoniae	Pseudomonas Species	Others (Proteus Mirabilis, Acinetobacter	

				Species, Escherichia Coli)	
16-30	52	53	09	15	129
31-45	55	40	13	05	113
46-60	62	58	14	10	144
>60	84	92	14	24	214
Total	253	243	50	54	600

The above table shows that the total number of infections increases with age, with the age group over 60 having the highest number of infections at 214 cases (35.67%), while the age group 31 to 45 has the lowest at 113 cases (18.83%).

Table 1 Antibiotics resistance in Klebsiella Pneumoniae

Antibiotics	Sensitive	Resistant	Total
Meropenem	202 (83%)	41 (17%)	243
Amikacin	198 (81%)	45 (19%)	243
Aztreonam	100 (41%)	143 (59%)	243
Piperacillin/tazobactam	188 (78%)	52 (22%)	240
Ceftazidime	2 (100%)	0 (0%)	2
Ciprofloxacin	97 (40%)	145 (60%)	242
Colistin	41 (100%)	00 (0%)	41
Ceftriaxone	69 (29%)	173 (71%)	242
Cefixime	55 (23%)	183 (77%)	238
Ampicillin	0 (0%)	243 (100%)	243

The table 6 describes the Results of the antimicrobial susceptibility testing of Klebsiella pneumoniae. Amongst the 243 isolates of K. pneumoniae, Ampicillin had 100% resistance as no case of the drug was sensitive, and the drug became completely ineffective. Resistance by Cefixime was 77 % (183/238 isolates), while 71 % (173/242 isolates) of Ceftriaxone were found to be resistant. These high resistance rates imply that first-line antibiotics can no longer be used to treat RTIs caused by K. pneumoniae in this region. On the other hand, several antibiotics were effective against K. pneumoniae. Similar to the other antipseudomonal drugs, sensitivity to Meropenem of 83% (202/243 isolates) was shown, as well as to Amikacin of 81% (198/243) and Piperacillin/Tazobactam of 78 % (188/240).

Table 2. ABR Against Ps. Aeruginosa

Antibiotics	Sensitive	Resistant	Total
Meropenem	216 (86%)	35 (14%)	251
Amikacin	208 (83%)	43 (17%)	251
Aztreonam	167 (66%)	85 (34%)	252
Piperacillin/tazobactam	226 (90%)	25 (10%)	251
Ceftazidime	173 (71%)	72 (29%)	245
Ciprofloxaci	151 (60%)	102 (40%)	253
Colistin	17 (100%)	00 (0%)	17
Ceftriaxone	0 (0%)	7 (100%)	7
Cefixime	0 (0%)	8 (100%)	8
Ampicillin	1 (11%)	8 (89%)	9

The sensitivity and resistance pattern shows that Piperacillin/Tazobactam Meropenem and amikacin are the most effective drugs against Ps. Aeruginosa. These are followed by Aztreonam and Ceftazidime. In contrast, drugs such as ampicillin, Cefixime, and ceftriaxone showed high levels of resistance against these microbes (see Table 4.7). Out of 253 P. aeruginosa isolates indicates that Piperacillin/Tazobactam, Meropenem and Amikacin remain frontline agents against this pathogen and that there is no overwhelming evidence for resistance to these agents at this time. Aztreonam and Ceftazidime also had moderate sensitivity (167 and 173 cases, respectively) and can be used as secondary options in the treatment protocols. P. aeruginosa, on the other hand, showed low susceptibility to a great many of the commonest antibiotics. Overall, 102 out of 253 cases (approximately 40%).

Discussion

The distribution of respiratory infections differs among populations and countries, depending on socioeconomic conditions, geographical and climatic. In this study, the overall of sputum culture positive of patients was 600 subject, 343 (57.2%) sputum samples were isolation-positive from male patients, This trend suggests that respiratory infections cause more damage in males than in females .This finding is comparable to result reported from Zagazig, Egypt, 50.4% (12) and South India, 55.3% (13). This difference could be due to geographical location, study period and socioeconomic condition of the study population. Age group distribution in RTI Patients indicated that majority of cases belongs to increased age range b/w 61-100 year**.**

PS. Aeruginosa 42.2% was the most frequent bacterial isolate followed by Klebsiella Pneumoniae 40.5%. Pseudomonas species 8.3 % and Proteus mirabilis Acinetobacter species Escherichia coli 9.0%.This finding is comparable to the study conducted in Assuit, Egyp,in which K. pneumoniae was 58% but P. aeruginosa was low 28%. (14).One study from Southern Ethiopia observed Pseudomonas species 7.6%, Escherichia coli 5.5% which are very high comparing to our study (15).The RTI cases were found increased in October, November, and December, and then came back to the level equivalent to all other months. In our study, Meropenem shows a high rate of sensitive 85%, Amikacin 82%, Aztreonam 54%, Piperacillin/tazobactam, 84%, Ceftazidime71%, Ceftriaxone 50%, Cefixime 28%), and Ampicillin 0.003%. The current study revealed that amongst the 243 isolates of K. pneumoniae, Ampicillin had 100% resistance as no case of the drug was sensitive, and the drug became completely ineffective. Resistance by Cefixime was 77 % while 71 % of Ceftriaxone were found to be resistant. These high resistance rates imply that first-line antibiotics can no longer be used to treat RTIs caused by K. pneumoniae in this region. On the other hand, several antibiotics were effective against K. pneumoniae. Similar to the other antipseudomonal drugs, sensitivity to Meropenem of 83% was shown, as well as to Amikacin of 81%and Piperacillin/Tazobactam of 78 % . In contrast our study few have reported varies in antibiotics resistance rate for instance study from Southern Ethiopia reported a high rate of sensitive to ciprofloxacin 91.7%, cefepime and cefoxitin 83.3% each and resistant to augmentin 56.6%, ceftazidime and ampicillin 52.8 (15),However other studies in Ethiopia showed, K. pneumoniae exhibit high rates of resistance to tetracycline 100% and augmentin 96.7% (16);local studies in sindh suggests antibiotic sensitivity, Cefixime and Ceftriaxone were effective in 83.9% and 85.7% of cases, respectively (17). Our study assessed the Relationship between the patient's age and the isolated organism with number of infections increases with age; the age group over 60 having the highest number of infections at 214 (35.67%), while the age group 31 to 45 has the lowest at 113 (18.83%) .Similarly, studies reported that elder age is a significant risk factor for LRTI, with the risk growing especially for people > 65 age(18).

Conclusion:

Antibiotics resistance to RTI in Sindh was observed, with a striking predominance of Pseudomonas aeruginosa and Klebsiella pneumoniae, particularly affecting elderly males. Colistin, Meropenem, and Piperacillin/Tazobactam were effective; however, first-line antibiotics such as Ampicillin and Cefixime exhibited high resistance in this study. The study emphasizes the importance of culture-based prescribing and highlights the need for improved

diagnostic capacity and AMR surveillance on a regional scale. Public education regarding antibiotic misuse was implemented, and future research in the areas of the rising threat of multidrug resistance and the opposing goal of achieving effective treatment outcomes is recommended

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