

# Prevalence and susceptibility patterns of bacteria causing respiratory tract infections in North Waziristan, Pakistan

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**Abstract:** Respiratory tract infections (RTIs) are the most common infectious diseases in humans and are the major cause of mortality and morbidity in Pakistan. These infections are the leading causes of consultations in primary care in Pakistan. Therefore, this study was aimed at determining bacterial pathogens of respiratory tract infections and the susceptibility patterns of bacterial isolates to antibiotics. The study was conducted between February, 2013 and March, 2014 in North Waziristan region of Pakistan. Sputum specimens were collected aseptically from 227 patients and cultured on the appropriate bacteriological media. Bacterial isolates were identified by biochemical tests and their antibiotics susceptibility patterns were determined by standard methods. Out of 227, various species of bacteria were isolated from 152 (75%) specimens. The prevalence of bacteria species isolated were as follows *Pseudomonas aeruginosa* (42.8%), *Streptococcus pneumoniae* (26.7%), *Corynebacterium diphtheria* (10.6%), *Staphylococcus aureus* (5.9%), *Proteus vulgaris* (4.6%), *Micrococcus* species (3.3%), *Klebsiella pneumoniae* (2.6%) and *Bacillus* species (2.6%). The susceptibility patterns varied among bacterial species depending on the antibiotics. For the susceptibility test 11 commercially available antibiotics against bacterial isolates were used. The results revealed that generally the bacterial isolates were susceptible to gentamicin (80.9%), meropenem (75%), ceftazidime (62.5%), cefotaxime (57.9%) and ceftriazone (57.9%) and resistant to penicillin (84.9%) and doxycycline (78.9%). The antibiotics gentamicin (100%), meropenem (100%), ceftriaxone (58.5%), ciprofloxacin (60%), trimethoprim (60%), ceftazidime (66.2%) and cefotaxime (64.6%) were observed effective against the *P. aeruginosa* isolates. The findings of our study provide significant information for empiric therapy of patients with RTIs in North Waziristan region of Pakistan.

**Keywords:** Respiratory tract infections; susceptibility patterns; bacterial isolates; antibiotics; North Waziristan.

## INTRODUCTION

Respiratory tract infections (RTIs) are diseases of the upper or lower respiratory tract, including pharyngitis, scarlet fever, diphtheria, epiglottitis, sinusitis, otitis externa, acute otitis media, acute pertussis, tuberculosis, bronchitis, and pneumonia (Ndip *et al.*, 2008; Adegboro, 2010). RTIs are not only restricted to the respiratory tract but also show systemic effects due to probable prolongation of infection or inflammation, persistence of bacterial toxins and abnormal function of lungs (Prescott *et al.*, 2005). Actually, the human respiratory tract normally contains flora of aerobic bacterial species including *Streptococcus*, *Neisseria* (in the nasopharynx), *Staphylococcus*, *Haemophilus* (in the anterior nares of the nose), *Corynebacterium*, Enteriobacteriaceae and many anaerobes especially *bacteroides* (Adegboro, 2010). However, bacteria in respiratory tract are usually harmless and beneficial to the human body, but they may cause diseases when introduced, by chance, in sterilized organs like sinuses or in immuno-compromised patients. Sometimes, RTIs are initiated by viruses and impaired

immunity cause bacteria super-infections in humans, especially with opportunistic pathogens usually inhabiting the human respiratory tract (Carroll, 2002; Motayo *et al.*, 2012).

RTIs are commonly prevalent both in children and in adults, and are considered important public health problems particularly in developing countries. In many developing countries, RTIs are recognized as the leading causes of death and cause approximately 3.5 million deaths in children each year (Baqui *et al.*, 2007). The management of patients suffering from RTIs is a persistent challenge for general practitioners due to difficulty in diagnosis and emergence of antibiotic resistance against antimicrobial agents.

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 (Zafar *et al.*, 2008). For a general practitioner the important diagnostic tools are medical history and physical examination of patients. The chest X-ray is recommended only for few patients. In general practice,

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microbiological investigations are rarely carried out in Pakistan. The World Health Organization (WHO) estimates that 2 millions of the children under five years of age die of pneumonia each year in Pakistan (Bryce *et al.*, 2005). In another study Najam and his colleagues reported that upper respiratory tract infections are more common among children in Pakistan (Najam *et al.*, 2000). Acute respiratory tract infections were remained the most common cause of consultations, total 8954 consultations were reported for acute cases accounting for 32% of the all consultations reported over a one year period in Federally Administrated Tribal Area (FATA) and Khyber Pakhtunkhwa regions of Pakistan (WHO, 2009).

North Waziristan in FATA is a mountainous region of northwest Pakistan with the population of about 0.6 million. The area has been described as a land of high and difficult hills with deep and rugged defiles. The average height of the North Waziristan hills is 1,500-2,500 meters above sea level. The climate is very cold in winters prevailing from October till April. The mean maximum and minimum temperatures during the month of January are 10 and -2 degrees Celsius, respectively (Khan, 2007). The cold weather of North Waziristan plays an important role in contribution of respiratory infections in the community. Moreover, according to the findings of the National Nutrition Survey in 2011, malnourishment among women and children has increased in North Waziristan over the last decade. Malnutrition has made children particularly vulnerable to diseases including diarrhea, dysentery, anemia, pneumonia and other respiratory tract infections in internally displaced people due to war against terrorism in this locality (Dawn, 2011).

To our knowledge, there is limited information on the various prevailing bacterial pathogens of RTIs and their antibiotic susceptibility patterns in population of North Waziristan, Pakistan. Therefore, this study was conducted to find out baseline information on the susceptibility patterns of bacteria causing RTIs in North Waziristan region of Pakistan.

### **Methodology**

#### *Sampling population*

The study population, were those patients with symptoms of RTIs who attended the Head Quarter Hospitals in Miranshah and Mirali, in North Waziristan between February, 2013 to March, 2014. Consent was obtained from each patient to participate in this study. The demographic data like age and gender was recorded. Only a single positive culture of each patient was included in the microbial analysis. The patients, who were on antibiotics in a week before the samples collected or patients, whose sputum smear positive for acid-fast bacilli were excluded. The Ethical Committee of Department of Microbiology, Kohat University of Science and Technology (KUST) approved this study.

### **Specimen collection and bacteriological analysis**

The sputum specimens were collected aseptically from all patients into well-labeled sterile, wide mouthed glass bottles with screw cap tops. The specimens were transported immediately to the Microbiology Laboratory of the Department of Microbiology, KUST, for analysis. Viscous, mucoid or purulent sputum specimens were considered for analysis. First Gram-stained smear of each specimen was examined microscopically then Ziehl Neelsen staining was performed. The bacterial pathogens were isolated using suitable bacteriological media such as MacConkey Agar, Blood Agar and Chocolate Agar. The plates were then incubated at 37°C for 24-48 hours.

The morphological, physiological, and biochemical features of the bacterial isolates were determined according to Bergey's Manual of Systematic Bacteriology (Sneath *et al.*, 1986).

### **Antimicrobial susceptibility testing**

Susceptibility tests were performed on significant bacterial isolates using the Kirby-Bauer disk diffusion method, following the recommendations of the Clinical and Laboratory Standard Institute (CLSI, 2012). Mullen-Hinton agar was prepared with addition of 5% sheep blood. A 0.5 McFarland turbidity standard equivalent bacteria suspension for inoculation was prepared and inoculated. Antibiotic disks (Oxoid Ltd. UK) were applied and the plate was incubated at 37°C for 48 hours. The following antibiotics were used: Meropenem (MEM, 100µg), Erythromycin (ERY, 15µg), Gentamicin (CN, 10µg), Ceftriaxone (CRO, 30µg), Ciprofloxacin (CIP, 5µg), trimethoprim-sulfamethoxazole (SXT, 25µg), Penicillin (PCN, 10µg), Ampicillin (AMP, 10µg), Doxycycline (DO, 30µg), Ceftazidime (CAZ, 30µg) and Cefotaxime (CTX, 30µg).

After 48 hours of incubation, the diameters of inhibition zones around the disks were measured by using a graduated ruler and results were interpreted according to CLSI guidelines.

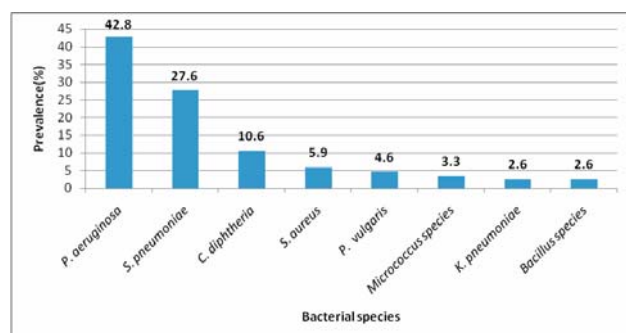
### **STATISTICAL ANALYSIS**

The Statistical Package for Social Science (SPSS) version 18.0 software was used for the descriptive statistical analysis of the data of the sample population. The frequencies and percentages were calculated for variables under study.

### **RESULTS**

Out of 257 specimens of patients analyzed, 152 (59.1%) had positive cultures. Among these 152 patients 103 (67.8%) were males and 49 (32.2%) were female. The prevalence of bacteria species isolated from sputum specimens of patients attending the Head Quarter

Hospitals in Miranshah and Mirali, in North Waziristan, is presented in fig. 1. Eight main species of bacteria were identified: *Pseudomonas aeruginosa* (42.8%), *Streptococcus pneumoniae* (26.7%), *Corynebacterium diphtheria* (10.6%), *Staphylococcus aureus* (5.9%), *Proteus vulgaris* (4.6%), *Micrococcus* species (3.3%), *Klebsiella pneumoniae* (2.6%) and *Bacillus* species (2.6%).



**Fig. 1:** Bacterial species detected from sputum specimens of patients with RTIs.

Table 1 shows the distribution of isolated bacterial species in patients with different age-groups. Of the 152 positive cultures recorded, the percentage of bacterial isolation in the various age-groups was 30.3% for the age-groups 1-16 and 65-80 years, 17.7 % for the age-group of 48-64 years, 14.5% for the age-group of 32-47 years and 7.2% for the age group 16-31 years. Among the isolated bacterial species *P. aeruginosa* and *S. pneumoniae* were found predominant isolates in all age groups.

The overall antibiotic susceptibility patterns of bacterial isolates as shown in table 2 revealed that the overall resistance rates were generally low for gentamicin (19.1%) and meropenem (25%), followed by ceftazidime (37.5%), cefotaxime (42.1%), ceftriazone (42.1%) and ciprofloxacin (43.4%). However, isolated bacterial pathogens were observed highly resistant to penicillin (84.9%) and doxycycline (78.9%), followed by ampicillin (64.5 %), erythromycin (60.5 %) and trimethoprim (52%). The antibiotic susceptibility patterns of individual bacterial species are shown in table 3. The variable susceptibility patterns were observed depending on the antibiotic and species of bacteria. The antibiotics meropenem (100%) and gentamicin (100%) exhibited maximum activity while ceftriaxone (58.5%), ciprofloxacin (60%) trimethoprim (60%), ceftazidime (66.2%) and cefotaxime (64.6%) were also observed effective against the *P. aeruginosa* isolates. Similarly ceftazidime (83.3%) and ciprofloxacin (78.6%) showed high activity while meropenem (59.5%), erythromycin (52.3%), gentamicin (59.5%), ceftriaxone (64.3%) and cefotaxime (64.3%) were also observed effective against *S. pneumoniae*. The susceptibility rate of meropenem, gentamicin and ceftriaxone was observed 56.3% for *C. diphtheria* isolates. The most affective antibiotic against

*S. aureus* isolates was ceftazidime (88.9%) followed by trimethoprim (77.8 %), erythromycin (66.7%), gentamicin (66.7%), cefotaxime (66.7%) and ceftriaxone (55.6%). *P. vulgaris* isolates were observed highly susceptible to gentamicin (100%) and comparatively less susceptible to trimethoprim (71.4%), meropenem (57.2%) and cefotaxime (57.1%). Similarly the isolates of *Micrococcus* species isolates were also observed to highly susceptible to gentamicin (100%) and comparatively less susceptible to cefotaxime (80%), meropenem (60%) and erythromycin (60%). The antibiotic trimethoprim (100%) exhibited maximum activity while gentamicin (75%) and cefotaxime (75%) exhibited good activity against *K. pneumoniae* isolates. Three antibiotics meropenem (75%), gentamicin (75%) and ciprofloxacin (75%) were observed equally effective against *Bacillus* species isolates.

## DISCUSSION

The pathogenic agents responsible for respiratory tract infections and their antibiotic susceptibility profiles vary between geographical locations (Egbagbe and Mordi, 2006; Egbe *et al.*, 2011). Moreover, updated knowledge of the prevailing bacterial pathogens of respiratory infections in defined population is required to monitor suspected bacterial pathogens, whereas determination of high risk population group is essential for implementation of preventive strategies. Moreover, monitoring of antibiotic susceptibility patterns of the etiological bacterial pathogens is required not only to guide the clinicians for the prescription of appropriate treatment but also necessary to monitor the trends of respiratory infections. Therefore, microbiological investigation is critical for both epidemiological and treatment purposes.

This study also focused on the prevalence and antibiotics susceptibility patterns of bacteria causing RTIs in population of North Waziristan region. Based on results obtained in this study, *P. aeruginosa* was the most common (42.8%) isolated pathogen among others. The result obtained in this study differ from what was reported in studies performed by Egbe *et al.* (2011) in Nigera and Siddalingappa *et al.* (2013) in India, where *S. pneumoniae* and *K. species* were the most frequent cause of RTIs respectively. However, in our study *S. pneumoniae* (27.6%) was the second predominant pathogen causing RTIs, followed by *C. diphtheria* (10.5%), *S. aureus* (5.9%), *P. vulgaris* (4.5%), *Micrococcus* species (3.3%), *K. pneumoniae* (2.6%) and *Bacillus* species (2.6%) as shown in fig. 1. Similar studies conducted in other regions of world reported variable profiles of bacterial pathogens causing RTIs (García-de-Lomas *et al.*, 2002; Ndip *et al.*, 2008; Kousalya *et al.*, 2010; Medella *et al.*, 2012).

The high prevalence of RTIs was recorded among young children (1-16 years, 30.3%) and elderly (65-80 years, 30.3%) as compared to young age patients (16-31years, 7.2%) and middle-age patients (32-47 years, 14.5%; 48-

**Table 1:** Distribution of isolated bacterial respiratory pathogens in relation to age of patients

Bacterial isolates	Age groups					Total	
	1-15	16-31	32-47	48-64	65-80	No.	%
	<i>P. aeruginosa</i>	21	6	6	14	18	65
<i>S. pneumoniae</i>	14	2	10	8	8	42	27.6
<i>C. diphtheria</i>	6	0	3	0	7	16	10.6
<i>S. aureus</i>	3	0	1	1	4	9	5.9
<i>P. vulgaris</i>	2	0	0	1	4	7	4.6
<i>Micrococcus</i> species	0	0	2	3	0	5	3.3
<i>K. pneumoniae</i>	0	3	0	0	1	4	2.6
<i>Bacillus</i> species	0	0	0	0	4	4	2.6
Total	46	11	22	27	46	152	100

**Table 2:** Overall antibiotic susceptibility patterns of respiratory tract isolates

Antibiotics	Antibiotic susceptibility patterns of bacterial isolates (N=152)	
	Resistance n (%)	Sensitive n (%)
Meropenem	38 (25)	114(75)
Erythromycin	92(60.5)	60(39.5)
Gentamicin	29(19.1)	123 (80.9)
Ceftriaxone	64(42.1)	88(57.9)
Ciprofloxacin	66 (43.4)	86(55.6)
Trimethoprim	79(52)	73(48)
Penicillin	129(84.9)	23(15.1)
Ampicillin	98(64.5)	54(35.5)
Doxycycline	120(78.9)	32(21.1)
Ceftazidime	57(37.5)	95(62.5)
Cefotaxime	64(42.1)	88(57.9)

64 years, 17.6%) in this study. Our findings are not in agreement with findings of another study conducted in Nigeria in which the highest prevalence of RTI was recorded among the age group 31 to 40 years (22.5%) and least among the age 11-20 and 61-70 years (5.0%), (Akingbade *et al.*, 2012). The reasons behind the high prevalence of bacterial isolates observed in this study among young children and elderly are malnutrition and cold climate of North Waziristan. These factors can lower the body's resistance, opening the way for endogenous infections in humans as previously suggested (Ndip *et al.*, 2008; Motayo *et al.*, 2012). The high prevalence of RTIs observed in this study among elder patients could be attributed to the fact that patients of this age group are usually immune-compromised (McEllistrem *et al.*, 2002).

The results of the antibiotic susceptibility study generally revealed that gentamicin (80.9%) and meropenem (75 %) were the most effective antibiotics against the isolates. Gentamicin exhibited highest activity (100%) against *P. aeruginosa*, *P. vulgaris* and *Micrococcus* species. Similarly, meropenem showed highest activity (100%) against *P. aeruginosa*. Overall the ceftazidime (62.5%), cefotaxime (57.9%) and ceftriazone(57.9%) were also observed effective against respiratory pathogens in our study. Ceftazidime exhibited better activity against *S.*

*aureus* (88.9%) and *S. pneumoniae* (83.3%) isolates while cefotaxime exhibited better activity against *Micrococcus* species (80%) isolates. Various studies from other parts of world also reported better susceptibility of antibiotics reported in our study against various respiratory infections pathogens. Okesola and Ige reported 83.3% susceptibility rates while Drusano and his colleagues reported 93% susceptibility rate against the RTIs pathogens to meropenem (Drusano *et al.*, 2000; Okesola and Ige, 2012). The results of the antibiotic susceptibility studies from India generally revealed that gentamicin, ceftazidime, cefotaxime and ceftriazone were the most effective antibiotics against the isolates (Kousalya *et al.*, 2010; Jyothsna *et al.*, 2010). A study from Cuba reported that bacterial pathogens causing LRTIs were sensitive to ceftriaxone, gentamicin, amikacin, meropenem, aztreonam and piperacillin/tazobactam and highly resistant to ampicillin (Medella *et al.*, 2012). Similarly another study from Nigeria reported ofloxacin, ciprofloxacin, cefuroxime, cetazidime, ceftriaxone and gentamicin showed moderate to high activity while sulfamethoxazole-trimetroprim, tetracycline, cloxacillin, and erythromycin were not active against any bacterial isolates causing LRTIs (Egbe *et al.*, 2011). Taura and his colleagues also from Nigeria reported that antibiotics Ceftriaxone, Ceftazidime, Ciprofloxacin, Ofloxacin,

**Table 3:** Antibiotic susceptibility patterns of isolated individual bacterial species

Bacterial isolates		Percentage of bacterial isolates sensitive and resistance to										
		MEM	ERY	CN	CRO	CIP	SXT	PCN	AMP	DO	CAZ	CTX
<i>P. aeruginosa</i>	R	0	64.6	0	41.5	40	40	90.8	61.5	86.2	33.8	35.4
	S	100	35.4	100	58.5	60	60	9.2	38.5	13.8	66.2	64.6
<i>S. pneumoniae</i>	R	40.5	47.7	40.5	35.7	21.4	76.2	88.1	61.9	71.4	16.7	35.7
	S	59.5	52.3	59.5	64.3	78.6	23.8	11.9	38.1	28.6	83.3	64.3
<i>C. diphtheria</i>	R	43.7	81.3	43.7	43.7	93.8	75	81.3	87.5	75	93.8	68.8
	S	56.3	18.7	56.3	56.3	6.2	25	18.7	12.5	25	9.2	31.2
<i>S. aureus</i>	R	66.7	33.3	33.3	44.4	55.6	22.2	66.7	55.6	77.8	11.1	33.3
	S	33.3	66.7	66.7	55.6	44.4	77.8	33.3	44.4	22.2	88.9	66.7
<i>P. vulgaris</i>	R	42.8	85.7	0	42.9	57.2	28.6	71.4	71.4	71.4	71.4	85.7
	S	57.2	14.3	100	57.1	42.8	71.4	28.6	28.6	28.6	28.6	14.3
<i>Micrococcus</i> species	R	40	40	0	60	80	60	60	60	60	60	20
	S	60	60	100	40	20	40	40	40	40	40	80
<i>K. pneumoniae</i>	R	50	75	25	50	50	0	75	75	75	50	20
	S	50	25	75	50	50	100	25	25	25	50	75
<i>Bacillus</i> species	R	25	75	25	75	25	50	75	50	100	50	100
	S	75	25	75	25	75	50	25	50	0	50	0

Gentamicin and Chloramphenicol showed activity on all the pathogens isolated from patients with RTIs (Taura *et al.*, 2013).

An important fact is that the preferred route of administration for gentamicin, meropenem, cefuroxime, cefotaxime and ceftriazone is by intramuscular injection or intravenous infusion, therefore these are less abused as compared to other orally administered antibiotics. Moreover, these antibiotics are broad-spectrum, therefore are effective against many aerobic and anaerobic bacteria. These factors may be responsible for the low rate of bacterial resistance observed for these antibiotics in our study and reported previously in various studies (Ndip *et al.*, 2008; Kousalya *et al.*, 2010; Okesola and Ige, 2012). Therefore, these may be drugs of choice in treating respiratory infections in North Waziristan Population.

Penicillin and doxycycline are prescribed for empiric treatment of many different types of infections caused by bacteria. Moreover, these antibiotics are well absorbed after oral administration; therefore they are clinically useful in the outpatient setting (Payne and Benninger, 2007). The high resistance to penicillin (84.9%) and doxycycline (78.9%) observed in this study and previously reported, thus suggesting a relationship between antibiotic use and the level of drug resistance encountered in this study as previously suggested in other studies (Kousalya *et al.*, 2010; Siddalingappa *et al.*, 2013; Regasa *et al.*, 2014). The reasons behind resistance of these drugs may be frequent prescription by physicians, abuse of drugs, free availability at counters in drug stores and indiscriminate and inappropriate use, are potential factors in emergence of resistant strains (Ndip *et al.*, 2008; Jafari *et al.*, 2009; Siddalingappa *et al.*, 2013)

## CONCLUSIONS

This study highlights the bacterial aetiology of RTIs in North Waziristan region of Pakistan and the susceptibility profiles of bacterial isolates. An overall prevalence of 75 % of RTIs was observed in this study. Patients of age groups 1-16 years and 65-80 years have a significantly higher prevalence of RTI compared with other age groups. *P. aeruginosa* is the most predominant bacterial isolates. Gentamicin, meropenem, ceftazidime, cefotaxime and ceftriazone were the effective antibacterial agents. The results of this study may be used to improve diagnosis of RTIs in clinical laboratories, to revise the empirical antibiotic prescriptions and treatment for RTIs in primary care may be considered in connection with the likely aetiology in the North Waziristan region. Ultimately, this study will help to improve patient management and to reduce the burden of respiratory infections in this region.

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