Antimicrobial susceptibility pattern of bacterial strains Isolated from Respiratory Specimens in Khwaja Yunus Ali Medical College Hospital

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ABSTRACT

Respiratory tract infection is the most common acute illness, ranging from uncomplicated infection like common cold to life threatening infections like epiglottitis and pneumonia. A retrospective study was done to know the prevalence of microorganisms causing respiratory tract infections and determine the susceptibility pattern of bacterial isolates from respiratory tract infection (RTIs).

The study was carried out for one year between January 2013 to December 2013. A total 145 different clinical samples (tracheal aspirate, throat swabs, sputum, oral swabs, nasal swabs and pleural fluid) were collected from patients who attended the hospital from various regions, mostly from North Bengal part of Bangladesh. All clinical specimens were collected aseptically from patients and cultured on the appropriate bacteriological media. All bacteriological isolates were identified by morphology, microscopy and biochemical studies and antimicrobial susceptibility tests were performed by standard methods. Out of 145 samples, 61 samples were positive growth with various pathogens. Out of 61 positive samples 65.57% were males and 34.43% were females. Most of the Prevalent pathogens were Staphylococcus aureus (42.62%) followed by Pseudomonas aeruginosa (26.23%), Escherichia coli (19.67%), Klebsiella pneumoniae (9.84%) and Streptococcus pneumoniae (1.64%).

23 different commercial antibiotics were used to determine the susceptibility pattern of bacterial isolates from respiratory tract infection (RTIs). The susceptibility profiles varied from one bacterial isolate to the other depending on the nature antibiotics. Most of the bacteria were highly (70% ≥) sensitive to Amikacin Ceftazidime, Gentamycin, Imipenem, Levofloxacin, Meropenem and Tobramycin; Moderately susceptible to Amoxycillin, Azithromycin, Ciprofloxacin, Doxycycline, Cefixime, Ceftriaxone and Piperacillin and least (< 20%) sensitive to Amoxiclav, Carbenicillin, Cephradine and Cloxacillin. These findings have clinical and epidemiological significance and it helps the studies on the identification of the causative pathogens and susceptibility pattern of pathogens in the respiratory tract infection as well as enhances the knowledge of clinicians to select best antibiotic therapy which ultimately become helpful for diagnosis and treatment of the patients.

Key Words: Respiratory infection, Antibiotics. Susceptibility, Resistance

INTRODUCTION

Respiratory tract infection is one of the most important infectious diseases of human and a leading cause of morbidity and mortality in many developing countries [1]. Nearly 50 million deaths are associated with RT infections in the worldwide in each year and RT infections occur in both community and heath care setting [2]. For instance, over 62 million persons suffer from cold annually in USA alone [3], while in the United Kingdom, about 8 million persons are infected by some forms of chronic lung diseases which now kill one in every five persons [4]. In Canada, respiratory disease is accountable for over 16% of deaths and 10% of hospitalizations [5]. In developing countries like

Bangladesh 30% of all patient's consultation and 25% percent of all pediatric admission are of acute respiratory tract infections [6] which ultimately cause 3.5 million deaths of children each year [7]. Although, fungi and viruses are occasional etiological agents, RTIs are predominantly caused by facultative anaerobes. Diverse groups of organisms are colonized in the respiratory tract, particularly upper respiratory tract [8]. Most common isolates in Upper Respiratory Tract Infection(URTI) are *Streptococcus pyogenes*, and other beta hemolytic streptococci, *Staphylococcus aureus*, *Moraxella*, *Hemophilus influenzae*, *Streptococcus pneumoniae* and *Corynebacterium diphtheria* [9]. Bacterial etiology of lower Respiratory tract infection(LRI) in children varies from *Streptococcus pneumonia* (8-61%), *Hemophilus influenzae* (1-15%), Gram negative bacilli (3-5%), atypical agents like Chlamydia (1-14%), Mycoplasma (7-24%[10, 11]. Viral pathogens are also associated with respiratory tract infections accounts for 44-49% of the cases[12]. The prevalence of various bacteria differs from region to region, different seasons and different age group[13].

The antibiotic resistance developed by microbes has raised serious debates and has been recognized as a substantial problem by global medicinal and research community [1]. Many factors play vital roles in the emergence of resistance from improper practice of antimicrobial agents, transmission of resistant bacteria from patient to patient and healthcare workers to patients and vice-versa, lack knowledge for appropriate judicious use of antimicrobial agents [14]. Furthermore, some antibiotics are used in both animal and human. All these factors together contribute to the unavoidable rise and emergence of resistance of bacteria.
Resistance of antimicrobial agents are common problems to the Respiratory Tract Infections (RTIs) for both adult and children in Bangladesh. As in other developing countries, resistance of common antibiotics is dramatically increasing in Bangladesh due to its improper use and prescription. Clinical studies have shown that many penicillin-resistant pneumococci are also resistant to chloramphenicol, and cephalosporins such as cefuroxime and ceftriaxone, thus limiting treatment options [15]. The resistance of antimicrobials in Respiratory Tract Infections (RTIs) increases the risk of the effectiveness of antibiotics that increase the morbidity and mortality rate and also increase the health cost to the patient. The present study was conducted to know the prevalence of microorganisms, causing respiratory tract infections and to determine the current antibiotic susceptibility pattern of these bacterial isolates. This will enable the clinicians to formulate rational antibacterial policy and further control the incidence of the disease.

MATERIAL & METHODS:

The research was carried out in the Microbiology Laboratory Service Department of Khwaja Yunus Ali Medical College Hospital, Sirajgonj, Bangladesh. The population studied was a heterogeneous population of different age group and sex type. Total 145 respiratory clinical samples were collected from patients who attended the hospital from various regions of Bangladesh. The study was carried out for one year between January 2013 to December 2013. All data were collected from the registered book of Microbiology Laboratory Service Department of KYAMCH which contains detail record of culture and sensitivity test of different clinical samples.

The specimens were collected aseptically from patients with respiratory tract infections (145). Samples were included tracheal aspirates (n= 65), Throat swabs (n= 43), Sputum (n= 25), Oral swabs (n= 3), Nasal swabs (n= 3) and Pleural fluid (n= 6).

Various types of differential media were used to identify the clinical isolates. All media were prepared according to the
manufacturer's guideline and sterilized at 121°C for 15 minutes at 15 lb pressure. The inoculum on the plate was streaked out for discrete colonies with a sterile wire inoculating loop. The culture plates were incubated at 37°C for 24 hours and observed for growth through the formation of colonies. All

the bacteria were isolated and identified using morphological, microscopy and biochemical tests following standard procedures described by Cheesbrough [16].

For antibiotic sensitivity test of the test strains, twenty-three commercial antibiotics (Oxoid, UK) were used through Kirby-Bauer disk diffusion method [17]. Muller Hinton agar was used and sensitivity test was accomplished as per recommendation of Clinical and Laboratory Standards Institute (CLSI) [18]. The commercial available antibiotic discs used for the study were Ampicillin (10 µg), Amoxycillin (10 µg), Amoxiclav (30 µg), Amikacin (30 µg), Azithromycin (15 µg), Cephradine (30 µg), Cefixime (05 µg), Cefuroxime (30 µg), Ceftazidime (10 µg), Ciprofloxacin (05 µg), Cloxacillin (05 µg), Gentamicin (10 µg), Ceftriaxone (30 µg), Carbenicillin (100 µg), Doxycycline (30 µg), Erythromycin (15 µg), Imipenem (10 µg), Levofloxacin (05 µg), Meropenem (10 µg), Penicillin G (10 µg), Piperacillin (100 µg), Cotrimoxazole (25 µg), and Tobramycin (10 µg). A lawn of test pathogen (1ml of a 24-hour peptone broth culture) was prepared by evenly spreading 100 µl inoculums on the agar plate. The antibiotic discs were gently and firmly placed on the agar plates, which were then left at room temperature for 1 hour to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 37°C for 24 hours. If an antimicrobial activity was present on the plates, it was indicated by an inhibition zone. The diameter of the inhibition zones was measured in millimeter at 24 hours using the electronic scale. An organism was interpreted as highly susceptible, intermediate or resistant by the size of ‘‘zone of inhibition’’. The intermediate readings were considered as resistant in the assessment of the data. The SPSS windows version 23.0 software was used to analyze the data.

RESULTS:

During the research period, a total 145 respiratory samples were collected of which 61 (42.07%) showed culture positive and others were appeared as culture negative. Positive growth samples were analyzed to identify pathogens and their antimicrobial susceptibility following standard guideline. Most common samples were tracheal aspirate (65), throat swabs (43) and sputum (25) followed by oral swabs (3), nasal swabs (3) and pleural fluid (6) (Table-1). Isolation rate of different bacteria within tracheal aspirate, throat swabs, sputum and oral swabs samples were 52.31%, 46.51%, 24.00% and 33.33% respectively. Nasal swabs and pleural fluid showed no growth. The overall prevalence of bacterial isolates was 23.45% in tracheal aspirate, 13.79% in throat swabs followed by 4.14% in sputum and 0.69% in oral swabs (Table 1). Our study showed, more male patients (65.57%) suffered from respiratory tract infection than did from female counterparts (34.43%) (Figure 1).

The most common organisms isolated were *Staphylococcus aureus 2***6** (42.62%), *Pseudomonas aeruginosa* **16** (26.23%), *Escherichia coli* **12** (19.67%), *Klebsiella pneumoniae* **6** (9.84%) and *Streptococcus pneumoniae* **1** (1.64%) (Figure-2).

Among them, *E coli* and *Pseudomonas aeruginosa* were predominantly isolated from tracheal aspirate with a frequency of 10 and 16 respectively (Table 2). On the other hand, *Staphylococcus aureus* was higher in throat swabs and most *Klebsiella pneumoniae* was in sputum with the occurrence of 17 and 3 respectively.

Antimicrobial susceptibility study of different respiratory isolates revealed that three antibiotics ampicillin, Erythromycin, and Cloxacillin are 100% resistant against *E coli*, *Pseudomonas aeruginosa, & Klebsiella pneumoniae* (Figure 3, 4, & 6). Other highly resistant antibiotics were carbenicillin & Cefuroxime against *E coli,* Amoxiclav, ampicillin, amoxicillin, Carbenicillin, and Cefixime against *Pseudomonas aeruginosa,* and ampicillin & Penicillin G against *Klebsiella pneumoniae.* For *E coli,* the most sensitive antibiotics were Ceftazidime (100%), Imipenem (100%), Meropenem (100%), Amikacin (70%) and Tobramycin (83.33%) (Figure 3). For *Pseudomonas* *aeruginosa*, higher sensitivities were found in Amikacin (93.73%), Imipenem (100%), Ceftazidime (100%), Levofloxacin (100%) and Meropenem (93.75%) (Figure 4). Most of the antibiotics used against *Staphylococcus aureus* were found to be highly susceptible (Figure 5). Noteworthy, amikacin, carbenicillin, amoxiclav, ampicillin, Doxycycline, and piperacillin were observed to be more than 85% susceptible against the pathogen. The most resistant antibiotics against *Staphylococcus aureus* were found Erythromycin (77.78%) and Cloxacillin (68.75%). Hundred percent sensitivity against *Klebsiella pneumoniae* were found in antibiotics, Amikacin, Amoxiclav, Ceftazidime, Gentamycin, Ceftriaxone, Cefuroxime, Imipenem, Meropenem, Levofloxacin, Picarcillin, and cotrimoxazole (Figure 6). All antibiotics tested against Streptococcus pyogenes were found fully susceptible except amoxicillin and Ceftazidime (Table 3)

DISCUSSION:

Our study demonstrates to evaluate the multidrug resistance among the bacteria causing respiratory tract infection in Khwaja Yunus Ali Medical College Hospital, Sirajgong, Bangladesh. In our study, a total of 61 samples out of 145 had positive growth; growth prevalence of specific organisms was *Escherichia coli* (19.67%), *Pseudomonas aeruginosa* (26.23%), *Staphylococcus aureus* (42.62%), *Klebsiella pneumoniae* (9.84%) and *Streptococcus pneumoniae* (1.64%) respectively. These findings were in agreement with other works[19]. In terms of frequency of isolates, the results were in accordance with those conducted in other countries such as China [20], Cameroun, South Africa[21], Japan [22], Israel [23]and Turkey [24].

Sex-related occurrence of pathogens reveals that, male subjects reported higher number of Pathogens (65.57%) compared to their female counterpart (34.47%). This prevalence is due to higher risk factors (e.g. smoking and alcoholism) of respiratory infections associated with males than females in our country. This is consistent with other studies [25].

In our study, *Escherichia coli* Showed higher sensitivity rate to Amikacin (70.00%), Ceftazidime (100.00%), Imipenem (100.00%), Meropenem (100%), and Tobramycin (83.33%) and comparatively moderate to lower sensitivity to Amoxycillin (66.67%), Gentamycin (66.67%), Cotrimoxazole (66.67%), Levofloxacin (66.67%), Azithromycin (57.14%), Doxycycline (50.00%), and Ciprofloxacin (45.45%).Similar pattern obtained from other studies [26, 27]

The study showed that *Pseudomonas aeruginosa* isolates were highly susceptible to antibiotics Ceftazidime (100.00%), Imipenem (100.00%), Levofloxacin (100.00%), Meropenem (93.33%), Piperacillin (80.00%), Tobramycin (75.00%), Amikacin (93.75%), and Gentamycin (75.00%). Lower susceptibility against the pathogen were found in case of Amoxycillin (11.11%), Amoxiclav (0.00%), Ampicillin (0.00%), Carbenicillin (0.00%), Cephradine (20.00%), Cefixime (0.00%), Doxycycline (25.00%), Erythromycin (0.00%), Cloxacillin (0.00%) and Cotrimoxazole (16.67%). The patterns are in line with the other study conducted in other countries [26].

In the study, *Staphylococcus aureus* isolates were found more susceptible than other respiratory pathogens to Amikacin (93.33%), Amoxiclav (100.00%), Ampicillin (87.50%), Carbenicillin (90.91%), Ceftazidime (85.71%), Cefuroxime (83.33%), Doxycycline (87.50%), Imipenem (75.00%), Meropenem (83.33%), Penicillin G (83.33%), and Piperacillin (100.00%), and other antibiotics showed sensitivity to below 70% such as Azithromycin (52.17%), Cefixime (54.55%), Erythromycin (22.22%), and Cloxacillin (31.25%). These outcome are consistent with other study[1].

Our study showed that *Klebsiella pneumoniae* was highly susceptible to Amikacin (100.00%), Amoxiclav (100.00%), Ceftazidime (100.00%), Gentamycin (100.00%), Ceftriaxone (100.00%), Cefuroxime (100.00%), Imipenem (100.00%), Levofloxacin (100.00%), Meropenem (100.00%), Piperacillin (100.00%), Cotrimoxazole (100.00%) and Tobramycin (100.00%); and least susceptible to Ampicillin (0.00%), Carbenicillin (0.00%), Cephradine (25.00%), Cefixime (50.00%), Erythromycin (0.00%), OB (0.00%) and Penicillin G (0.00%). All are a similar susceptibility profile compared with other studies [27].

Streptococcus pneumoniae showed more susceptible to Amikacin (100.00%), Amoxiclav (100.00%), Azithromycin (100.00%), Carbenicillin (100.00%), Cephradine (100.00%), Ciprofloxacin (100.00%), Gentamycin (100.00%), Ceftriaxone (100.00%) and Tobramycin (100.00%) and least susceptible to Amoxycillin (0.00%) and Cefixime (0.00%).

The overall study showed that, most of the organisms had higher susceptibility to Amikacin Ceftazidime, Gentamycin, Imipenem, Levofloxacin, Meropenem and Tobramycin; moderate susceptibility to Amoxycillin, Azithromycin, Ciprofloxacin, Doxycycline, Cefixime, Ceftriaxone and Piperacillin and least effectivity to Amoxiclav, Carbenicillin, Cephradine and Cloxacillin; which is also comparable to susceptibility patterns reported from previous studies[28]

ABBREVIATIONS

AK= Amikacin, AMC=Amoxiclav, AML= Amoxycillin, AMP= Ampicillin, AZM= Azithromycin, CAR=Carbenicillin, CAZ= Ceftazidime, CE= Cephradine, CFM= Cefixime, CIP= Ciprofloxacin, CN= Gentamycin, CRO= Ceftriaxone, CXM= Cefuroxime, DO= Doxycycline, E= Erythromycin, IPM= Imipenem, LEV= Levofloxacin, MEM= Meropenem, OB= Cloxacillin, P= Penicillin G, PRL= Piperacillin, SXT= Cotrimoxazole, TOB= Tobramycin.

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PLACE OF STUDY:

Department of Laboratory Services, Khwaja Yunus Ali Medical College Hospital & Microbiology Laboratory of Khwaja Yunus Ali University, Enayetpur, Sirajgonj, Bangladesh.

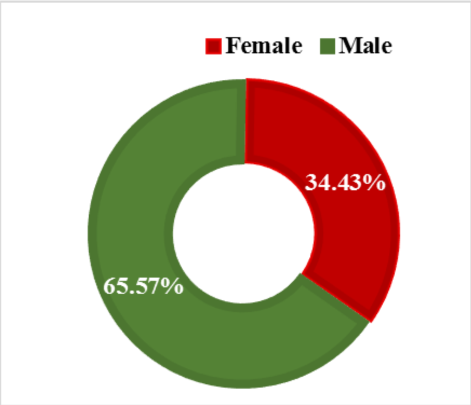

Figure 1: Distribution of respiratory pathogens among sex

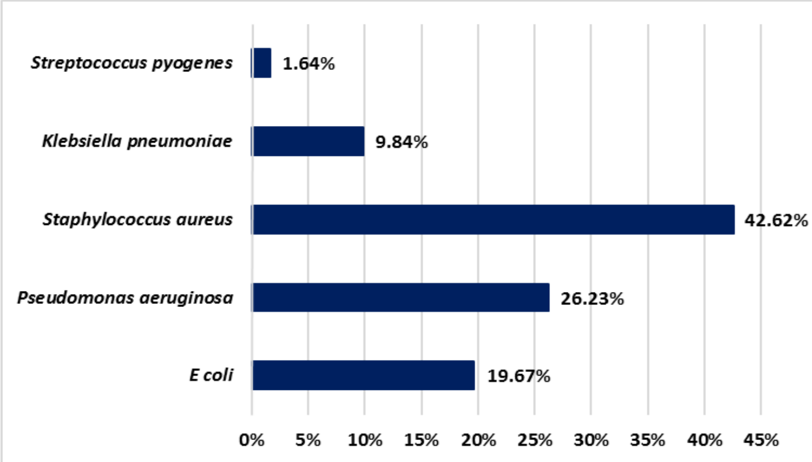

Figure 2. Prevalence of respiratory pathogens among all positive samples

Type of Specimens	Total	No. of positive isolates	Prevalence (%)	
			Within specimen	Within total
Tracheal aspirate	65	34	52.31	23.45
Throat swabs	43	20	46.51	13.79
Sputum	25	6	24.00	4.14
Oral swabs	3	1	33.33	0.69
Nasal swabs	3	0	0.00	0.00
Pleural fluid	6	0	0.00	0.00
Total	145	61	42.07	42.07

Table 1. Isolation rate of respiratory pathogens from various respiratory samples

Type of specimen	Isolate					Total
	E coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Streptococcus pyogenes*	
Trachaeal aspirate	10	16	6	2	0	**34**
Throat swabs	1	0	17	1	1	**20**
Sputum	1	0	2	3	0	**6**
Oral swabs	0	0	1	0	0	**1**
Total	**12**	**16**	**26**	**6**	**1**	**61**

Table 2. Distribution of Bacterial pathogens in different respiratory samples

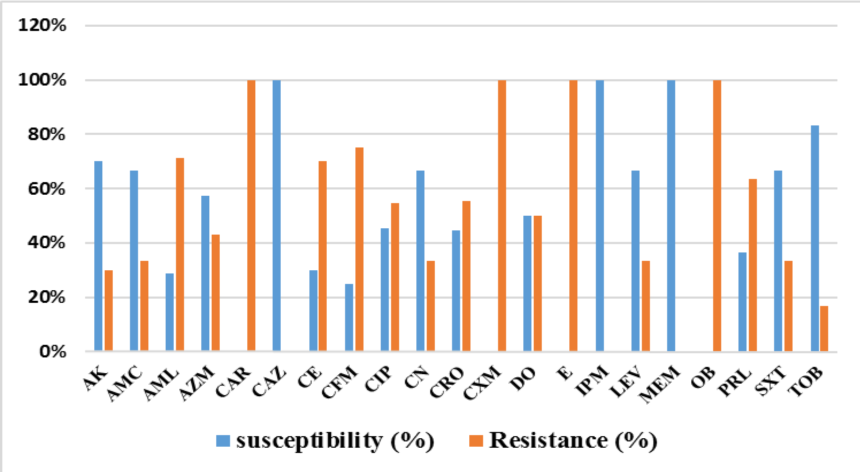

Figure 3: Activity pattern of respiratory *E coli* against different antibiotics

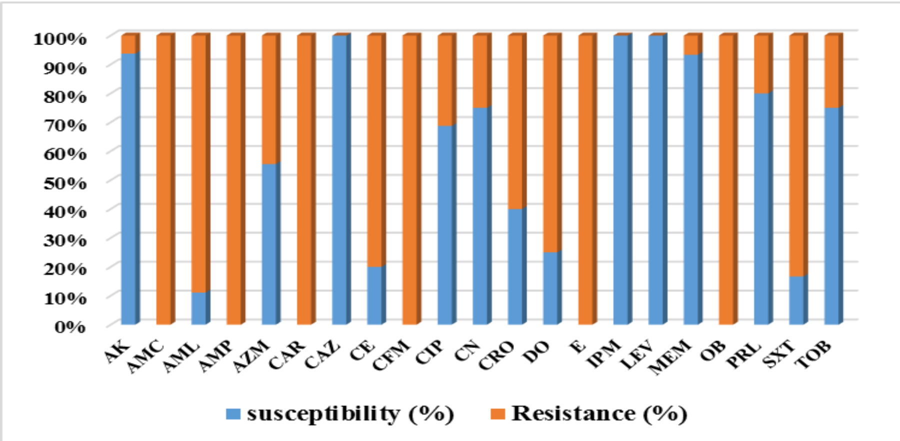

Figure 4: Activity pattern of respiratory *Pseudomonas aeruginosa* against different antibiotics

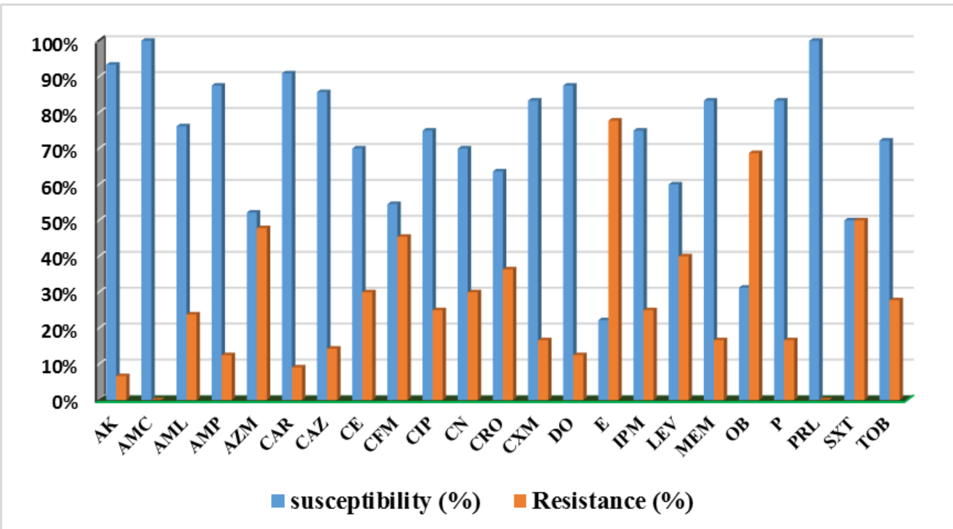

Figure 5: Activity pattern of respiratory *Staphylococcus aureus* against different antibiotics

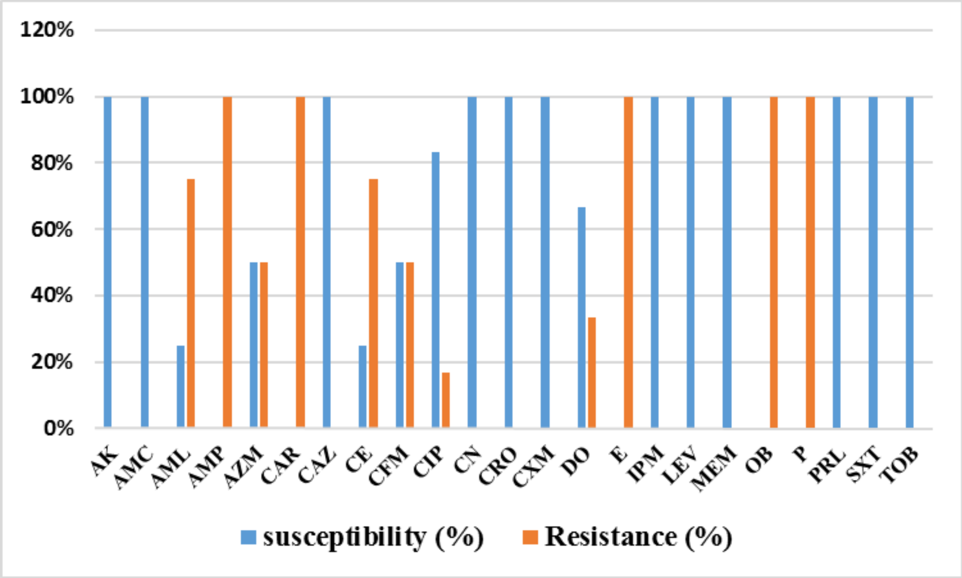

Figure 6: Activity pattern of respiratory *Klebsiella pneumoniae* against different antibiotics

Antibiotics	**Tested**	**Resistant**	**Sensitive**	**susceptibility (%)**
AK	1	0	1	100
AMC	1	0	1	100
AML	1	1	0	0
AZM	1	0	1	100
CAR	1	0	1	100
CE	1	0	1	100
CFM	1	1	0	0
CIP	1	0	1	100
CN	1	0	1	100
CRO	1	0	1	100
TOB	1	0	1	100

Table 3: Activity pattern of respiratory *Streptococcus pyogenes* against different antibiotics

REFERENCES:

1. Manikandan C. and A. Amsath, "Antibiotic susceptibility of bacterial strains isolated from patients with respiratory tract infections".
2. Zafar A., Hussain Z., Lomama E., Sibille S., Irfan S. and Khan E., "Antibiotic susceptibility of pathogens isolated from patients with community-acquired respiratory tract infections in Pakistan--the active study". Journal of Ayub Medical College, 2008. 20(1): p. 7.
3. Boyce J. A., Assa'ad A., Burks A.W., Jones S.M., Sampson, H.A. Wood, R.A., et al., "Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel". J Allergy Clin Immunol, 2010. 126(6 Suppl): p. S1-58.
4. Jarrold I. and N. Eiser, "P235 BLF and BTS 'Ready for home' survey of patients admitted to hospital with COPD: the hospital experience". Thorax, 2010. 65(Suppl 4): p. A175-A175.
5. DeSanto K.R., "Public Health Agency of Canada. Journal of Consumer Health on the Internet", 2011. 15(2): p. 196-206.
6. Rahman M.M. and A.M. Rahman, "Prevalence of acute respiratory tract infection and its risk factors in under five children". Bangladesh Med Res Counc Bull, 1997. 23(2): p. 47-50.
7. Hart C.A. and S. Kariuki, "Antimicrobial resistance in developing countries". Bmj, 1998. 317(7159): p. 647-50.
8. Lopez A.D. and C.C. Murray, "The global burden of disease, 1990-2020". Nat Med, 1998. 4(11): p. 1241-3.
9. Greenberg D. and E. Leibovitz, "Community-acquired pneumonia in children: from diagnosis to treatment". Chang Gung Med J, 2005. 28(11): p. 746-52.
10. Karchmer A.W., "Increased antibiotic resistance in respiratory tract pathogens": PROTEKT US--an update". Clin Infect Dis, 2004. 39 Suppl 3: p. S142-50.
11. Amin R., Hoque A.M.W., Khan R.F. and Rahman M., "Considering respiratory tract infections and antimicrobial sensitivity: An exploratory analysis". Growth. 101: p. 26.3.
12. Matsumoto A., Hashimoto K., Kawasaki Y. and Hosoya M., "A challenge to appropriate antibiotic use in children with respiratory infections: a 5-year single-institution experience". Fukushima J Med Sci, 2011. 57(2): p. 33-45.
13. Nweze E., Ezute S. Emeka Nweze CC., Ogbonna CC. and Eze C., "Bacteria etiological agents causing respiratory tract infections in children and their resistance patterns to a panel of ten antibiotics". Asian Pacific Journal of Tropical Disease, 2012. 1(2): p. 18-23.
14. Aly M. and H.H. Balkhy, "The prevalence of antimicrobial resistance in clinical isolates from Gulf Corporation Council countries". Antimicrob Resist Infect Control, 2012. 1: p. 26.
15. Islam Amirul, Mazumdar Reaz Mohammad, Fakrudin MD., Islam Saiful, Nipa MN., Iqbal A., and Bhuiyan Habibur R., "Multiple antibiotic resistant bacteria on fruit from different markets of Chittgong city in Bangladesh". Bang. Res. Publ. J, 2010. 4(4): p. 342-350.
16. M., C., "Medical Laboratory Manual for Tropical Countries, Microbiology". (Tropical Health Technology/Butterworth and Co. Ltd., Cambridgeshire/Kent), 2006. Vol. 2.
17. Bauer A.W., Kirby W.M. Sherris J.C., and Turck M., "Antibiotic susceptibility testing by a standardized single disk method". Am J Clin Pathol, 1966. 45(4): p. 493-6.
18. Performance standards for antimicrobial susceptibility testing. 16th Informational Supplement. Clinical and Laboratory Standards Institute. , 2006. M 100-S17.
19. El-Mahmood AM., Isa H., Mohammed A., and Tirmidhi AB., "Antimicrobial susceptibility of some respiratory tract pathogens to commonly used antibiotics at the Specialist Hospital, Yola, Adamawa State, Nigeria". J Clin Med Res, 2010. 2(8): p. 135-142.
20. An-hua Wu, Nan Ren, Xi-mao Wen, Xia-yun Yi, Xun Huang, Xiu-hua Xu, Yu-xiu and Gong A., "Nosocomial Infection Point-Prevalence Survey: Results and Analysisof 193 Hospitals in China in 2001 [J]". Chinese Journal of Nosoconmiology, 2002. 8.
21. Liebowitz L.D., M. Slabbert, and A. Huisamen, "National surveillance programme on susceptibility patterns of respiratory pathogens in South Africa: moxifloxacin compared with eight other antimicrobial agents". J Clin Pathol, 2003. 56(5): p. 344-7.
22. Bartlett J.G. and L.M. Mundy, "Community-acquired pneumonia". New England Journal of Medicine, 1995. 333(24): p. 1618-1624.
23. Williams John V., Harris Paul A., Tollefson Sharon J., Halburnt-Rush Lisa L., Pingsterhaus Joyce M., Edwards Kathryn M., Wright Peter F. and Crowe Jr. James E., "Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children". New England Journal of Medicine, 2004. 350(5): p. 443-450.
24. Kyaw Moe H., Lynfield Ruth, Schaffner William, Craig Allen S., Hadler James, Reingold Arthur, Thomas Ann R., Harrison Lee H., Bennett Nancy M., and Farley Monica M, "Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant Streptococcus pneumoniae". New England Journal of Medicine, 2006. 354(14): p. 1455-1463.

25. Panda S., B.P. Nandini, and T. Ramani, "Lower respiratory tract infection-bacteriological profile and antibiogram pattern". International Journal of Current Research and Review, 2012. 4(21): p. 149.
26. Okesola A. and O. Ige, "Trends in bacterial pathogens of lower respiratory tract infections". Indian Journal of Chest Diseases and Allied Sciences, 2008. 50(3): p. 269.
27. Manikandan C. and A. Amsath, "Antibiotic susceptibility of bacterial strains isolated from patients with respiratory tract infections". International Journal of Pure and Applied Zoology, 2013. 1(1): p. 61-9.
28. Sharmin Sohey, Alamgir Farhana, Fahmida M. and Saleh Ahmed Abu, "Antimicrobial sensitivity pattern of uropathogens in children". Bangladesh Journal of Medical Microbiology, 2010. 3(2): p. 18-22.