ORIGINAL ARITCLE

Antimicrobial Susceptibility Pattern of Bacterial Strains Isolated from Respiratory Specimens in Khwaja Yunus Ali Medical College Hospital

Md. Babul Aktar [1*], Md. Shakhaowat Hossain[2], Dr. Abdullah Akhtar Ahmed[3]

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. The retrospective experiment was performed to understand the prevalence of microorganisms affecting the respiratory infections and find out the sensitivity patterns of bacterial isolates from respiratory tract infections (RTIs). This experiment was done for one year from January 2013 to December 2013. A total 145 different clinical samples (tracheal aspirate, throat swab, sputum, oral swab, nasal swab, and pleural fluid) were collected from patients who attended the hospital from various regions, mostly from North Bengal part of Bangladesh. All the clinical samples were collected following appropriate aseptic techniques from patients and cultivated on the suitable 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%). Twenty-Three (23) different commercial antibiotic discs were applied to measure the sensitivity pattern of bacterial isolates from respiratory tract infection (RTIs). The susceptibility profiles varied from one bacterial isolate to the other depending on the nature of 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 increase awareness of clinicians to select best antibiotic therapy which ultimately become effective to diagnose and treat the affected patients.

***Key Words:** Respiratory infection, Antibiotics, Susceptibility, Resistance*

1. INTRODUCTION

Respiratory tract infection is one of the communicable diseases of human and major cause of morbidity and mortality rate in 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 example, more than 62 million people suffer from cold yearly in USA alone [3], whereas in the United Kingdom, nearly 8 million people are affected from some kinds of chronic lung diseases which now kill one in every five persons [4]. In Canada, respiratory disease is responsible for more than 16% of deaths and 10% of hospital admissions [5]. In developing countries like Bangladesh 30% of all patient's consultation and 25% of all pediatric admission are of acute respiratory tract infections [6] which ultimately brings about 3.5 million deaths of children every year [7].

Although, fungi and viruses are occasional etiological agents, RTIs are predominantly caused by facultative

*1 **Lecturer**, Department of Microbiology, Khwaja Yunus Ali University, Enayetpur, Sirajgonj-6751, Bangladesh.*
*2 **Assistant Professor & Head,** Department of Microbiology, Khwaja Yunus Ali University, Enayetpur, Sirajgonj-6751, Bangladesh.*
*3 **Professor & Head, Department of Microbiology,** Khwaja Yunus Ali Medical College & Hospital, Enayetpur, Sirajgonj-6751, Bangladesh*
*** Correspondence to:** E-mail: babul127micro@gmail.com*

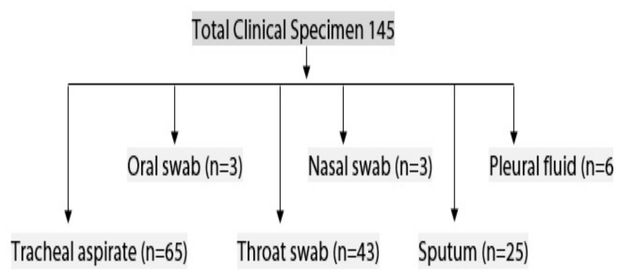
anaerobes. Diverse groups of organisms are colonized in the respiratory tract, particularly upper respiratory tract [8]. The most common isolates found in Upper Respiratory Tract Infection (URTI) are typically *Streptococcus pyogenes* and other beta hemolytic streptococci as well as *Staphylococcus aureus*, *Hemophilus influenzae*, *Streptococcus pneumoniae*, *Corynebacterium diphtheria*, and *Moraxella* [9]. Bacterial etiology of lower Respiratory tract infection(LRI) in children varies from *Streptococcus pneumoniae* (8-61%), *Hemophilus influenzae* (1-15%), Gram negative bacilli (3-5%), atypical agents like *Chlamydia* sp. (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 like improper practice of antimicrobial agents, lack of knowledge for appropriate application of antimicrobial substances, transmission of resistant bacteria from patient to patient and from patients to healthcare workers and vice-versa [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 substance is one of the major health issues in Bangladesh, especially in the treatment of Respiratory Tract Infections (RTIs) for both adult and children. As in other developing countries, resistance of common antibiotics is dramatically increasing in Bangladesh due to its improper use and prescription. Clinical researches have revealed that a number of penicillin-resistant pneumococci are also resistant to chloramphenicol, and cephalosporins such as cefuroxime and ceftriaxone, thus limiting treatment options [15]. The reduced susceptibility of antimicrobial agent in Respiratory Tract Infections (RTIs) poses the greater risk of the efficacy of antibiotics that steps up the morbidity and mortality rate and also intensifies the health cost to the patient. The purpose of this study was to understand the prevalence of microorganisms, causing respiratory tract infections and to find out the trend of antibiotic sensitivities of these bacterial isolates. This will enable the clinicians to formulate rational antibacterial policy and further control the incidence of the disease.

2. 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. A total 145 respiratory clinical samples were collected from patients who attended the hospital from various regions of Bangladesh. The experiment was done for one year, from January 2013 to December 2013. All data were collected from the registered book of Microbiology Laboratory Service Department of KYAMCH which contained detailed records of culture and sensitivity profile of different clinical specimens.



Flow Chart: Total Respiratory Samples Collected

To identify the clinical isolates, various types of selective and differential media were used. All media were prepared according to the manufacturer's guideline and sterilized at 121°C for 15 minutes at 15 lb pressure. To obtain discrete colonies, streak plate method was done by using a sterile wire inoculating loop. The culture plates were incubated at 37°C for 24 hours and monitored for growth through the development of colonies. Isolation and identification of all bacterial isolates were done by morphological, microscopy and biochemical tests complying with standard practices 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]. To determine the susceptibility pattern, Muller Hinton agar was used, and relevant test was done as per the guidelines 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 placed on the agar plates carefully and firmly, which were then kept at room temperature for 1 hour to allow the antibiotics to diffuse into the agar medium. The plates were then kept at 37°C for 24 hours in incubator. Any type of antimicrobial activity was observed by the visible zone of inhibition. The diameter of the inhibition zones was measured in millimeter at 24 hours using the electronic scale. Using the size of "zone of inhibition" the organism was defined as highly susceptible, intermediate or resistant to relevant antimicrobial agents. 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.

3. Results

During the research period, a total 145 respiratory samples were collected of which 61 (42.07%) showed culture positive and others appeared as culture negative. Positive growth samples were examined using standard guideline to identify pathogens and their antimicrobial sensitivity. Most common samples were tracheal aspirate (65), throat swab (43) and sputum (25) followed by oral swab (3), nasal swab (3) and pleural fluid (6) (Table-1).

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

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

Isolation rate of different bacteria within tracheal aspirate, throat swab, sputum and oral swab samples were 52.31%, 46.51%, 24.00% and 33.33% respectively. Nasal swab and pleural fluid showed no growth. The overall prevalence of bacterial isolates was 23.45% in tracheal aspirate, 13.79% in throat swab followed by 4.14% in sputum and 0.69% in oral swab (Table 1). Our study showed, more male patients (65.57%) suffered from respiratory tract infection than did from female counterparts (34.43%) (Figure 1).

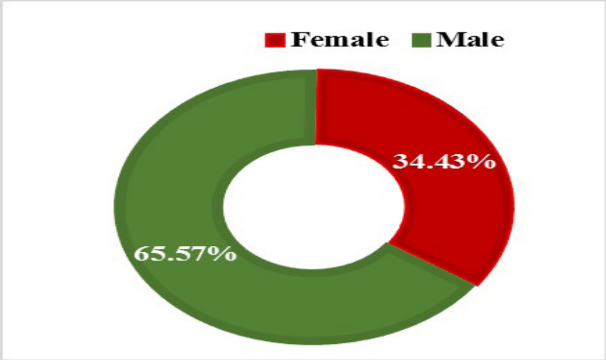


Figure 1: Distribution of respiratory pathogens among sex

The most common organisms isolated were *Staphylococcus aureus* **26** (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).

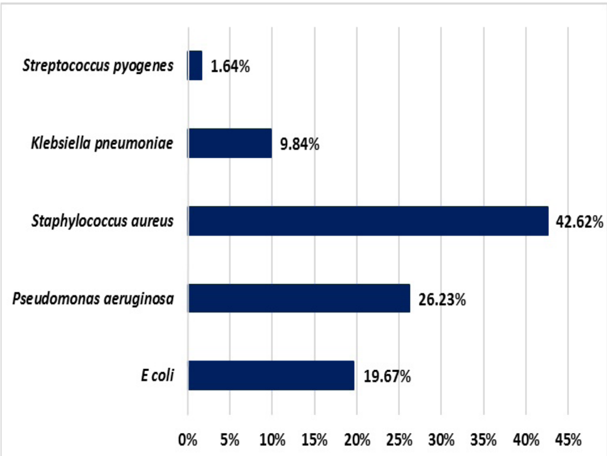


Figure 2. Prevalence of respiratory pathogens among all positive samples

Among them, *E. coli* and *Pseudomonas aeruginosa* were predominantly isolated from tracheal aspirate with a frequency of 10 and 16 respectively (Table 2).

Table 2. Distribution of bacterial pathogens in different 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 swab	1	0	17	1	1	20
Sputum	1	0	2	3	0	6
Oral swab	0	0	1	0	0	1
Total	12	16	26	6	1	61

On the other hand, *Staphylococcus aureus* was higher in throat swab 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 to *E. coli, Pseudomonas aeruginosa*, & *Klebsiella pneumoniae* (Figure 3, 4, & 6).

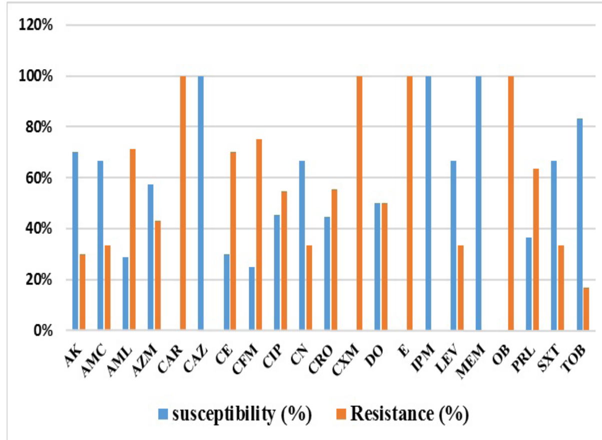


Figure 3: Activity pattern of respiratory *E. coli* against different antibiotics

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).

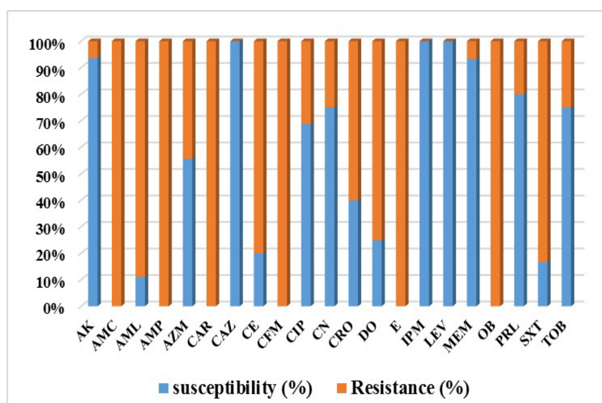


Figure 4: Activity pattern of respiratory *Pseudomonas aeruginosa* against different antibiotics

Most of the antibiotics used against *Staphylococcus aureus* were found to be highly susceptible (Figure 5).

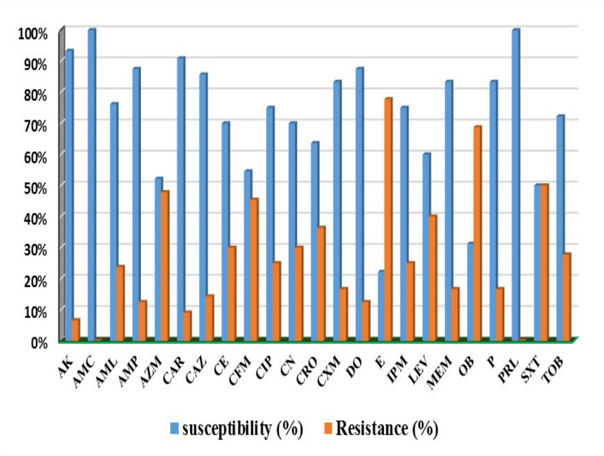


Figure 5: Activity pattern of respiratory *Staphylococcus aureus* against different antibiotics

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).

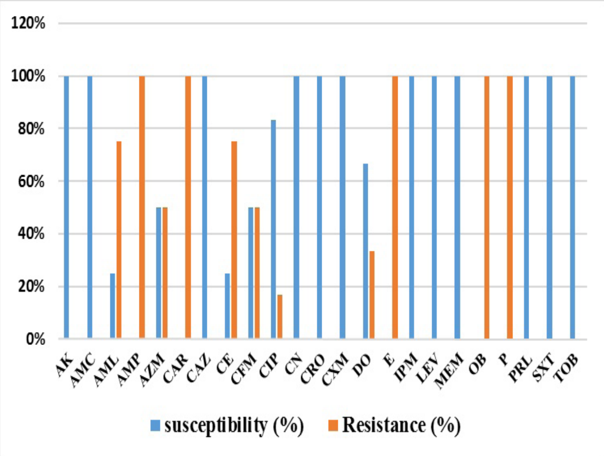


Figure 6: Activity pattern of respiratory *Klebsiella pneumoniae* against different antibiotics

All antibiotics tested against Streptococcus pyogenes were found fully susceptible except Amoxicillin and Ceftazidime (Table 3).

Table 3: Activity pattern of respiratory *Streptococcus pyogenes* 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

4. 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 outcomes aligned with other studies [19]. In terms of frequency of isolates, the findings were in agreement with those performed 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 exposure to environment pollution 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%). This trend of antimicrobial susceptibility is also supported by the other studies [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 outcomes 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%), Cloxacillin (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.

Acknowledgment

We would like to thank Mr. Mohammad Yusuf, Chairman of the Board of Trustees of Khwaja Yunus Ali University and Director of Khwaja Yunus Ali Medical College Hospital for the facilities provided to carry out the work. We are also grateful to Mr. Md. Abdul Karim, and Mr. Md. Mazharul Islam, Medical Technologist of the Department of Laboratory Services of Khwaja Yunus Ali Medical College Hospital for their assistance in laboratory work.

Place of study

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

References

1. Manikandan C, Amsath A. Antibiotic susceptibility of bacterial strains isolated from patients with respiratory tract infections. *Int. J. Pure Appl. Zool.* 2013;1(1): p. 61-69.

2. Zafar A, Hussain Z, Lomama E, Sibille S, Irfan S, 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 JA, Assa'ad A, Burks AW, 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, Eiser N. 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 KR. Public Health Agency of Canada. Journal of Consumer Health on the Internet. 2011;15(2): p. 196-206.

6. Rahman MM, Rahman AM. 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 CA, Kariuki S. Antimicrobial resistance in developing countries. *Bmj*. 1998;317(7159): p. 647-650.

8. Lopez AD, Murray CC. The global burden of disease, 1990-2020. *Nat Med*. 1998;4(11): p. 1241-1243.

9. Greenberg D, Leibovitz E. Community-acquired pneumonia in children: from diagnosis to treatment. *Chang Gung Med J*. 2005;28(11): p. 746-752.

10. Karchmer AW. Increased antibiotic resistance in respiratory tract pathogens: PROTEKT US--an update. *Clin Infect Dis*. 2004;39 Suppl 3: p. S142-150.

11. Amin R, Hoque AW, Khan RF, Rahman M. Considering respiratory tract infections and antimicrobial sensitivity: An exploratory analysis. *Mal. J. Microbiol.* 2009;5(2): p. 109-112.

12. Matsumoto A, Hashimoto K, Kawasaki Y, 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, 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, Balkhy HH. The prevalence of antimicrobial resistance in clinical isolates from Gulf Corporation Council countries. *Antimicrob Resist Infect Control*. 2012; 1: p. 26.

15. Islam A, Mazumdar RM, Fakrudin MD, Islam S, Nipa MN, Iqbal A, *et al*. 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. Cheesbrough M. Medical Laboratory Manual for Tropical Countries, Microbiology. (Tropical Health Technology/Butterworth and Co. Ltd., Cambridgeshire/Kent). 2006; Vol. 2.

17. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 1966; 45(4): p. 493-496.

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, 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 W, Ren N, Xi-mao W, Xia-yun Y, Huang X, Xiu-hua X, *et al*. A Nosocomial Infection Point-Prevalence Survey: Results and Analysisof 193 Hospitals in China in 2001. *Chinese Journal of Nosoconmiology*. 2002-2008.

21. Liebowitz LD, Slabbert M, Huisamen A. 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. 344-347.

22. Bartlett JG, Mundy LM. Community-acquired pneumonia. *New England Journal of Medicine*. 1995; 333(24): p. 1618-1624.

23. Williams JV, Harris PA, Tollefson SJ, Halburnt-Rush LL, Pingsterhaus JM., Edwards KM *et al.* 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 MH, Lynfield R, Schaffner W, Craig AS., Hadler J, Reingold R, *et al.* 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, Nandini BP, Ramani T. Lower respiratory tract infection-bacteriological profile and antibiogram pattern. *International Journal of Current Research and Review*. 2012;4(21): p. 149.

26. Okesola A, Ige O. 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, Amsath A. Antibiotic susceptibility of bacterial strains isolated from patients with respiratory tract infections. *International Journal of Pure and Applied Zoology*.2013;1(1): p. 61-69.

28. Sohey S, Farhana A, Fahmida M, Abu SA. Antimicrobial sensitivity pattern of uropathogens in children. *Bangladesh Journal of Medical Microbiology*. 2010;3(2): p. 18-22.