ORIGINAL ARTICLE
IN INFECTIOUS DISEASES

Bacterial profile and antibiotic susceptibility pattern of adult lower respiratory tract infections in Colombo, Sri Lanka

Nadeesha Amarasinghe[1], Muthulingam Athavan[2], Deepal Jayamanne[3], Yamuna Rajapakshe[4], Aflah Sadikeen[4], Kirthi Gunasekara[4], Amitha Fernando[4], Lilani Karunanayake[5]

Affiliations:

[1] M.D., Central Chest Clinic, Colombo, Sri Lanka
[2] M.D., Senior Registrar in Respiratory Medicine, Central Chest Clinic, Colombo, Sri Lanka
[3] M.D., Health Informatics, Ministry of Health, Colombo, Sri Lanka
[4] M.D., Consultant Chest Physician, Central Chest Clinic, Colombo, Sri Lanka
[5] M.D., Consultant Microbiologist, Medical Research Institute, Colombo, Sri Lanka.

Corresponding author:

Dr. Nadeesha Amarasinghe, Central Chest Clinic, Colombo, Sri Lanka.
E-mail: amaranadee@gmail.com

Abstract

Introduction: Lower respiratory tract infections (LRTIs) remain the deadliest communicable disease around the world. This study was conducted to identify the bacterial etiology of LRTIs among patients who attended the Central Chest Clinic in city of Colombo, Sri Lanka and their antibiotic susceptibility profile to enable clinicians to take decisions on effective empirical antibiotics.
Methods: Sputum samples were collected from 1,372 patients over the age of 18 years with suspected LRTIs during the year 2015. The samples were collected and processed according to standard laboratory procedures at the microbiology laboratory of the Medical Research Institute of Sri Lanka.
Results: Most of reports (58%) were from patients diagnosed with infective exacerbations of chronic lung diseases. Out of all sputum cultures processed, 404 (29.4%) resulted positive for pathogenic bacterial organisms. Coliforms (n = 176, 43.6%), and *Pseudomonas aeruginosa* (n = 117, 29%) were the most common isolated bacteria, followed by *Moraxella* (n = 47, 11.6%), *Haemophilus influenzae* (n = 23, 5.7%), and *Streptococcus pneumoniae* (n = 18, 4.4%). The two most common bacteria isolated showed a high sensitivity for co-amoxyclav, quinolones, 3rd generation cephalosporins, carbapenems and aminoglycosides, while coliforms were highly resistant (98%) to ampicillin. *S. pneumoniae* showed a high resistance for penicillin (67%) and erythromycin (61%), while *Haemophilus* showed a good sensitivity to co-amoxyclav (96%). There was no significant correlation between rainfall and proportions of coliforms (r = - 0.152, P = 0.638) and *Pseudomonas* (r = 0.271, P = 0.395) during the year.
Discussion and Conclusion: In our study, the most predominant pathogens recovered from LRTIs were *P. aeruginosa* and coliforms (*Klebsiella* spp.) as Gram negative, and *S. pneumoniae* as Gram positive bacteria. Co-amoxyclav, 3rd generation cephalosporins, quinolones and all second line antibiotics tested were the most efficient antibiotics in treatment of LRTIs, differently from ampicillin, erythromycin and penicillin that were not efficient antibiotics in treating this disease in our locality.

KEY WORDS: Antibiotic susceptibility; antibiotic resistance; bronchiectasis, chronic obstructive pulmonary disease; lower respiratory tract infections; Sri Lanka.

Riassunto

Introduzione: Le infezioni delle basse vie respiratorie restano le malattie trasmissibili più letali nel mondo. Questo studio è stato condotto per identificare l'eziologia batterica delle infezioni delle basse vie respiratorie tra i pazienti del Central Chest Clinic, nella città di Colombo, Sri Lanka ed il profilo di sensibilità agli antibiotici per dare ai clinici la possibilità di prendere decisioni per un efficace antibiotico-terapia su base empirica.
Metodi: Colture di espettorato sono state raccolte da 1.372 pazienti con più di 18 anni e con un sospetto di infezione delle basse vie respiratorie durante l'anno 2015. I campioni sono stati raccolti e processati secondo le procedure di laboratorio standard presso il laboratorio microbiologico del Medical Research Institute dello Sri Lanka.
Risultati: La maggior parte dei referti (58%) proveniva da pazienti con una diagnosi di riacutizzazione acuta di malattie polmonari croniche. Di tutte le colture di espettorato esaminate, 404 (29.4%) sono risultate positive per batteri patogeni. I coliformi (n = 176, 43.6%), e lo *Pseudomonas aeruginosa* (n = 117, 29%) sono stati i batteri più frequentemente isolati, seguiti da *Moraxella* (n = 47, 11.6%), *Haemophilus influenzae* (n = 23, 5.7%) e da *Streptococcus pneumoniae* (n = 18, 4.4%). I due batteri più frequentemente isolati hanno evidenziato un elevata sensibilità per co-amoxicillina, chinolonici, cefalosporine di terza generazione, carbapenemi ed aminoglicosidi, mentre i coliformi sono risultati altamente resistenti (98%) all'ampicillina. Lo *S. pneumoniae* ha mostrato un'elevata resistenza alla penicillina (67%) ed all'eritromicina (61%), mentre l'*Haemophilus* ha mostrato una buona sensibilità alla co-Amoxicillina (96%). Non è stata evidenziata una significativa correlazione tra la piovosità e la proporzione di coliformi (r = -0.152, P = 0.638) e di *Pseudomonas* (r = 0.271, P = 0.395) durante l'anno.
Discussione e Conclusioni: Nel nostro studio i patogeni predominanti nelle infezioni delle basse vie aeree respiratorie sono stati *P. aeruginosa* ed i coliformi (*Klebsiella* spp.) come Gram negativi, e lo *S. pneumoniae* tra i batteri Gram positivi. Co-amoxicillina, le cefalosporine di terza generazione, i chinolonici e tutti gli antibiotici di seconda linea testati sono stati i più efficienti nel trattamento di queste infezioni, a differenza dell'ampicillina, dell'eritromicina e della penicillina che non sono risultati efficaci nel trattamento di questa malattia nella nostra località.

TAKE-HOME MESSAGE

Empirical antibiotic regimens should be revised with novel data on etiological agents and the susceptibility patterns. Continuous surveillance is essential as pathogens and susceptibility patterns change over time.

Competing interests - none declared.

Cite this article as: Amarasinghe N, Athavan M, Jayamanne D, Rajapakshe Y, Sadikeen A, Gunasekara K, Fernando A, Karunanayake L. Bacterial profile and antibiotic susceptibility pattern of adult lower respiratory tract infections in Colombo, Sri Lanka. J Health Soc Sci. 2018;3(1):27-36

DOI 10.19204/2018/bctr3

Received: 16/01/2018 Accepted: 03/02/2018 Published: 15/03/2018

INTRODUCTION

Lower respiratory tract infections (LRTIs) remain the deadliest communicable disease and the 3rd leading cause of death around the world, after ischaemic heart and cerebrovascular diseases [1]. In developing countries management of LRTIs is difficult in both children [2] and adults [3], especially due to the issues associated with identification of the etiological agents and selection of appropriate antibiotics. LRTIs in adults include lower respiratory tract infections, acute bronchitis, influenza, suspected or definite community-acquired pneumonia, acute exacerbation of chronic obstructive pulmonary disease (COPD) and bronchiectasis [4].

The etiology of LRTIs cannot be determined clinically; moreover, it can vary based on age, gender and season. Moreover, many variations in bacterial profiles and antibiotic susceptibility patterns were identified geographically depending upon the antibiotic pressure on that locality [3, 5, 6].

Bacterial resistance to the effects of antibiotics is an increasing problem around the world. Multiresistant microorganisms, which in developed countries would result in the selection of an alternative treatment, in poor countries may cause infections that are untreatable [7]. Moreover, resistance surveillance data from parts of the developing world remain poor. Relatively few surveillance data are available for countries in South-East Asia [8].

The ANSORP study revealed high levels of penicillin resistance in S. pneumoniae in Vietnam and Thailand, intermediate rates in Singapore and lower rates in Indonesia and Malaysia. Low rates of penicillin nonsusceptibilty have been reported in India, Bangladesh and Pakistan. In contrast, 41% of isolates from Sri Lanka were reported to be penicillin intermediate [9]. Furthermore, Cotrimoxazole resistance is common in the Indian subcontinent and has been reported in over 50% of isolates in India and Bangladesh [10].

The majority of patients attending the Central Chest Clinic in Colombo City, Sri Lanka are adults (> 18 yrs), already diagnosed with pre-existing chronic lung diseases such as bronchiectasis, COPD, interstitial lung disease (ILD) and bronchial asthma (BA) and are frequently affected by infective exacerbations leading to LRTIs. Offending pathogens and the susceptibility pattern recovered in LRTIs of such patients is probably different to the general population.

Monitoring and identifying antibiotic resistance mechanisms in bacteria causing LRTIs is important to determine the most appropriate agents for initial empirical antimicrobial therapy and to target efforts to reduce inappropriate antibiotic use. In developing countries, mortality from LRTIs is high and limited therapeutic options and poorly regulated prescribing practices are likely to fuel the spread of resistance [8]. To our knowledge, there are little data available about bacteria etiological agents causing lower respiratory tract infections and their resistance patterns in Sri Lanka. Therefore, our study was aimed at describing the pathogenic bacteria profile and related antibiotic susceptibility pattern of patients affected by LRTIs, to enable clinicians to decide which is the most appropriate empirical antibiotics before the sputum cultures, serologies and antibiotic susceptibility reports are available.

METHODS

A descriptive study was carried out at Central Chest Clinic (CCC) with the collaboration of the microbiology laboratory of Medical Research Institute of Sri Lanka, at Colombo City. All sputum cultures and antimicrobial sensitivity tests reports of adult patients (aged >18 years) with signs and symptoms of LRTIs, during the year 2015, were recorded. Clinical records of the patients were cross checked by physicians of CCC to confirm the clinical presentation and record the coexisting chronic lung diseases [11].

Processing of samples

Sputum samples collected from the patients suspected with LRTIs were sent to the microbiology laboratory of the Medical Research Institute to be processed. Gram staining was performed initially to purulent or muco-purulent portion of the samples to identify the predominant intracellular microorganisms. The epithelial cell to pus cell ratio was calculated to decide on quality of the sample. Samples were inoculated in Blood, Chocolate and McConkey agar culture plates (Oxoid UK) and incubated at 37° C for 24 - 48 hours [12]. Bacitracin discs (10 units) and Optochin discs (5 μg) (Oxoid UK) were placed on primary and secondary inoculations to screen *Haemophilus influenzae* and *Streptococcus pneumoniae*, respectively. Emergent colonies were identified by the morphology of colonies, gram staining, and a panel of biochemical tests according to standard laboratory procedures. API kits were used for final confirmation of the isolates [13–15].

Antibiotic susceptibility testing

Antibiotic susceptibility testing was done by disc diffusion method on Mueller Hinton Agar (MHA), and Mueller Hinton Blood Agar plates. Ampicillin, co-amoxyclav, penicillin, cefuroxime, ceftriaxone, cefotaxime, ceftazidime, erythromycin, gentamycin, ciprofloxacin, levofloxacin, tetracycline and co-trimoxazole were tested as first line antibiotics. Meropenem, imipenem, cefoperazone sulbactam, ticarcillin, piperacillin tazobactam, netilmycin, amikacin, chloramphenicol, vancomycin and clindamycin were tested as second line antibiotics for relevant pathogens. Plates were incubated at 37° C for 16-18 hours (overnight). Diameters of the zones of inhibition were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Reference isolates *Haemophilus influenzae* ATCC 49247, 49766, *Escherichia coli* ATCC 25922, 35218, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC BAA 977, *Enterococcus faecalis* ATCC29212, *Streptococcus pneumoniae* ATCC 49619, and *Klebsiella pneumoniae* ATCC 700603 were used for quality control testing.

Statistical analysis

Statistical analysis was carried out using the statistical software for social sciences (SPSS) version 22.0. Frequency of isolated pathogens and the susceptibility rates were determined. Frequency of isolation of predominant pathogens in each month was compared with the temperature and rainfall pattern of the Colombo district during the year [16] and the correlation was assessed by using Spearman's correlation coefficient. The statistical significance cut-off was set at $P < 0.05$.

Ethical approval

Our study was conducted after obtaining patients' informed consent and ethical clearance from the ethical committee of the Medical Research Institute, Sri Lanka.

RESULTS

As shown in Table 1, 404 (29.4%) out of 1,372 samples processed yielded positive cultures for pathogens, of which mono-microbial growth was found in 99.5% (n = 402) and mixed pathogens were revealed in 0.5% (n = 2) of them. Mean age of the studied population was 58.7 (SD = 13) years. Out of all positive reports, 235 (58%) were of patients diagnosed with chronic lung diseases including bronchiectasis (n = 163), bronchial asthma (n = 28), COPD (n = 21), lung fibrosis following tuberculosis (n = 16) and interstitial lung diseases (n = 7). There were 225 (55.6%) male patients and 179 (44.3%) female patients with growth of pathogens. *Pseudomonas aeruginosa* was the most common (n = 117, 29%) gram negative pathogen isolated, although coliforms accounted for the highest total score as a group (n = 176, 43.6%). Out of 176 coliforms, 75 (42.6%) isolates had the colony morphology of *Klebsiella* spp. However, complete speciation of the coliform group was not carried out due to limited facilities in the laboratory. Five coliform isolates produced extended-spectrum beta-lactamases (ESBL). Among gram-positive isolates, *S. pneumoniae* was the predominant pathogen (n = 18, 4.4%). Antibiotic susceptibility profiles of bacterial isolates as first line and second line antibiotic panels are illustrated in Tables 2 and 3, respectively. *Pseudomonas aeruginosa* showed a high susceptibility for quinolones,

Table 1. Distribution of total bacterial isolates.

Bacteria-Gram stain	Bacterial species/group	Number (*n*)	Percentage (%)
Gram-negative *n* = 376 (93.1%)	Coliforms (*n* = 176, 43.6%) *Klebsiella* spp Other coliforms	176 75 101	43.6 18.6 25
	P. aeruginoasa	117	29.0
	Moraxella spp	47	11.6
	H. influenzae	23	5.7
	Acinetobacter spp	14	3.4
	Burkholderia cepacia	1	0.2
Gram–positive *n* = 28 (6.9%)	*S. pneumoniae*	18	4.4
	Groups A/C Streptococci	8	2.0
	S. aureus	2	0.5

Table 2. Antibiotic susceptibility pattern of coliforms, *P. aeruginosa*, *Moraxella* spp, *H. influenzae* and *S. pneumoniae* to first line antibiotic panels.

Antibiotic	Antibiotic susceptibility -Percentage (%)				
	Coliforms (*n* = 176)	***P. aeruginosa*** (*n* = 117)	***Moraxella*** (*n* = 47)	***H. influenzae*** (*n* = 23)	***S. pneumoniae*** (*n* = 18)
Ampicillin	1.5	-	-	73.9	-
Co-amoxyclav	73.2	-	95.7	95.6	-
Penicillin	-	-	-	-	33.3
Cefuroxime	56.8	-	-	-	100
Ceftriaxone	-	-	-	94.7	-
Cefotaxime	76.3	-	-	100	100
Ceftazidime	80.4	89	-	-	-
Erythromycin	-	-	40.0	-	38.9
Gentamycin	-	84.5	-	-	-
Ciprofloxacin	80.1	82	83	100	-
Levofloxacin		68	-	-	-
Tetracycline	-	-	57.4	-	-
Co-trimoxazole		-	20.9	50	27.3

Table 3. Antibiotic susceptibility pattern of Coliforms, *P. aeruginosa*, *Moraxella* spp, *H. influenzae,* and *S. pneumoniae* to second line antibiotic panels.

Antibiotic	Antibiotic susceptibility -Percentage (%)				
	Coliforms (*n* = 176)	***P. aeruginosa*** (*n* = 117)	***Moraxella*** (*n* = 47)	***H. influenzae*** (*n* = 23)	***S. pneumoniae*** (*n* = 18)
Meropenem	95	93.7	-	100	-
Imipenem	99	92.7		-	-
Cefoperazone s.*	-	83.3		-	
Ticarcillin c.*	84.8	80.7		-	-
Piperacillin t.*	83.3	-	-	-	-
Netilmicin	93.8	88.5			
Amikacin	95	87.1	-	-	-
Chloramphenicol	-	-	-	100	-
Vancomycin	-	-	-	-	100
Clindamycin	-	-	-	-	-

*s-sulbactam *c-clavulanate * t-tazobactam

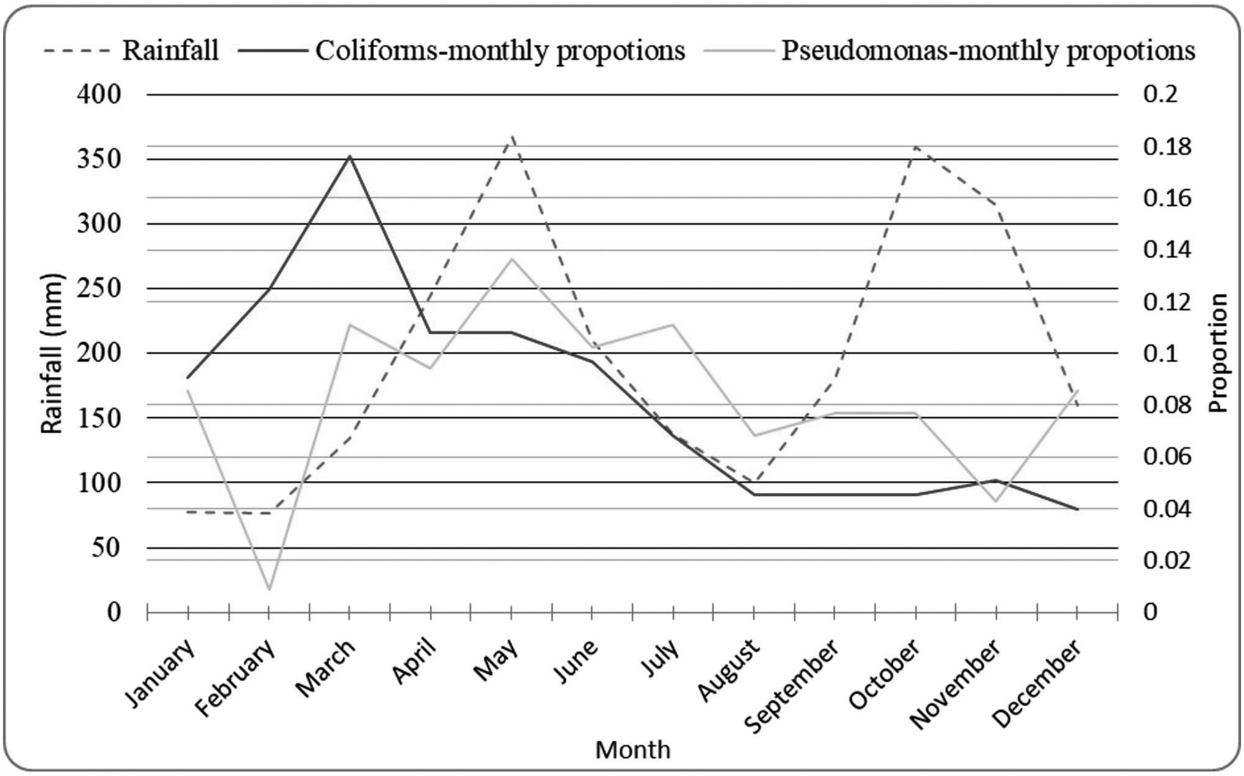

Figure 1. Rainfall pattern and isolation of coliforms and *Pseudomonas* during the year.

carbapenems and aminoglycosides, while all pathogens demonstrated high susceptibility rates (> 80%) for second line antibiotic panels tested. As shown in Figure 1, frequency of isolation of main pathogens during the months of the year was compared with the temperature and rainfall pattern of Colombo district during the year. There was no significant association between the rainfall and proportions of coliforms (r = -0.152, P = 0.638) and *Pseudomonas* (r = 0.271, P = 0.395).

DISCUSSION AND CONCLUSIONS

The primary objective of this study was to ascertain the current prevalence of bacteria causing LRTIs among patients who attended the Central Chest Clinic in Colombo City, Sri Lanka and to identify their antibiotic susceptibility pattern. Pathogens were recovered from 29% of the total specimens and this figure is within the range (18.9% - 59.4%) of previous reports [5, 17–19]. Assumption of antibiotics before obtaining culture samples by our clinic may probably explain this finding. In our research, a high percentage of monomicrobial growths were identified compared to previous studies [3]. Gram negative bacterial pathogens including *P. aeruginosa* and coliforms (*Klebsiella* spp.) played a significant role in causing LRTIs among patients attending our center, in agreement with previous studies [3, 17, 18, 20]. Presence of chronic lung diseases in the majority of patients and recurrent exposures to antibiotics during infective exacerbations, which produce alterations in the normal flora may have facilitated infections from such opportunistic pathogens [3]. However, *S. pneumoniae* remains the predominant pathogen among gram positives, which is in agreement with past research showing that it is a predominant pathogen in community-acquired LRTIs [21-24].

In our study, we also observed a higher prevalence of resistance of *S. pneumoniae* to erythromycin (macrolide) than that reported in 2001 in Sri Lanka [9]. Over the past years, antimicrobial resistance among *S. pneumoniae* has raised dramatically worldwide. By the early 1990s, penicillin-resistant clones of *S. pneumoniae* spread rapidly across the world [21]. Recently, it was reported that

15 to 30% of *S. pneumoniae* worldwide are multidrug-resistant (MDR) [25].
Amoxicillin differs from ampicillin only by the presence of a hydroxyl group in the benzene side chain, while its *in vitro* activity is identical to that of ampicillin [26]. As coliform group revealed a very high resistance for ampicillin, use of both amoxicillin and ampicillin as empirical antibiotics would not be a good choice for this cohort of patients. Pseudomonas isolates demonstrated good susceptibility rates against tested antibiotics compared to the susceptibility rates demonstrated by Khan et al. in 2015 [3]. Edirisinghe et al. (2009) have revealed high frequency rates of isolation of coliforms during the period May-August, which are the months of South West Monsoon season in Sri Lanka [20]. Conversely, in our study common isolates did not show a such association with rainy seasons.
In conclusion, the most predominant pathogens recovered from LRTIs in patients attending Central Chest Clinic, in Colombo City, capital of Sri Lanka, were *P. aeruginosa* and coliforms (*Klebsiella* spp.) as Gram negative, and *S. pneumoniae* as Gram positive bacteria. Co-amoxyclav, 3rd generation cephalosporins, quinolones and all second line antibiotics tested were the most efficient antibiotics in treatment of lower bacterial respiratory tract infections, differently from ampicillin, erythromycin and penicillin that were not efficient antibiotics in treating this disease in our locality.
Our research has some implications for future research. Data from our study could be useful to revise empirical antibiotic regimens, which should be focused by physicians on etiological agents and antibiotic susceptibility patterns, to prevent the emergence of resistant and/or multidrug resistant bacteria in LRTIs. Continuous surveillance based on local data is essential as pathogens and susceptibility patterns change over time [21].

Acknowledgements

Authors acknowledge the staff of the Central Chest Clinic and Microbiology Laboratory of the Medical Research Institute for the assistance given in numerous ways to complete this study.

References

1. World Health Organization (WHO). The top 10 causes of death. Geneva: WHO;2017 [cited 2018 Jan 02]. Available from: http://www.who.int/mediacentre/factsheets/fs310/en/.
2. Niederman MS, Krilov LR. Acute lower respiratory infections in developing countries. Lancet. 2013;381(9875):1341–1342.
3. Khan S, Priti S, Ankit S. Bacteria etiological agents causing lower respiratory tract infections and their resistance patterns. Iran Biomed J. 2015;19(4):240–246.
4. Woodhead M, Blasi F, Ewig S, Garau J, Huchon G, Ieven M. Guidelines for the management of adult lower respiratory tract infections. Eur Respir J. 2005;26:1138–1180.
5. Ozyilmaz E, Akan OA, Gulhan M, Ahmed K, Nagatake T. Major bacteria of community-acquired respiratory tract infections in Turkey. Jpn J Infect Dis. 2005;58(1):50–52.
6. Goel N, Chaudhary U, Aggarwal R, Bala K. Antibiotic sensitivity pattern of gram negative bacilli isolated from the lower respiratory tract of ventilated patients in the intensive care unit. Indian J Crit Care Med. 2009;13(3):148–151. doi:10.4103/0972-5229.58540.
7. Vila J, Pal T. Update on antibacterial resistance in low-income countries: factors favoring the emergence of resistance. Open Infect Dis J. 2010;4:38–54.
8. Felmingham D, Feldman C, Hryniewicz W, Klugman K, Kohno S, Low DE, et al. Surveillance of resistance in bacteria causing community-acquired respiratory tract infectious. Clin Microb Infect. 2002;8(Suppl 2):12–42.
9. Song JH, Lee NY, Ichiyama S, Yoshida R. Hirakata Y, Fu W. Spread of drug-resistant Streptococcus pneumoniae in Asian countries: Asian Network for Surveillance of Resistant Pathogens (ANSORP) Study. Clin Infect Dis. 1999 Jun;28(6):1206–1211.
10. Thacker SB. Surveillance. In: Gregg MB, ed. Field Epidemiology. Oxford: Oxford University Press;1996.
11. Amarasinghe N, Athavan M, Rajapakshe Y, Fernando A, Karunanayaka L. Pathogenic bacteria isolated from sputum samples and their antibiotic susceptibility pattern in adult patients treated at Central Chest Clinic, Sri Lanka. Respirology. 2017;22 (Suppl 3):270. doi:10.1111/resp.13207_494.
12. Bridson EY. The Oxoid Manual. 9th Edition, Oxoid limited: Basingtoke, England; 2006.
13. Barrow GI, Felthen RKA. Cowan and Steel's Manual for Identification of Medical Bacteria,3rd edition. Cambridge: Cambridge University Press; 1993.
14. Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie & McCartney Practical Medical Microbiology, 14th edition. New York: Churchill Livingstone; 1996.
15. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Color Atlas and Text book of Diagnostic Microbiology, 6th edition. Philadelphia, Pennsylvania: Lippincott-Raven Publishers; 2006.
16. Colombo Climate & Temperature. [Online]; Colombo Climate Table 2009-2014 [cited 2017 Jan 03]. Available from: http://www.colombo.climatemps.com.
17. Christopher AE, Casimir N, Richard O. Microbiology of Lower Respiratory Tract Infections in Benin City , Nigeria. Malays J Med Sci. 2011;18(2):27–31.
18. Egbagbe EE, Mordi RM. Aetiology of Lower Respiratory Tract Infection in Benin City, Nigeria. J Med Biomed Res. 2006;5(2):22–27.
19. Okesola AO, Ige OM. Trends in bacterial pathogens of lower respiratory infections. Indian J Chest Dis Allied Sci. 2007;50(3):270–272.
20. Edirisinghe LU, Kalukottage P, Maziama MNN, Silva PV. A retrospective observational study on the sputum samples received by the Department of Microbiology, Teaching Hospital, Karapitiya in the year 2007. Galle Med J. 2009;14(1):10–14.
21. Agmy G, Mohamed S, Gad Y, Farghally E, Mohammedin H, Rashed H. Bacterial Profile, Antibiotic Sensitivity and Resistance of Lower Respiratory Tract Infections in Upper Egypt. Med J Hematol Infect

Dis. 2013;5(1):e2013056. doi:10.4084/MJHID.2013.056.

22. Liu YN, Chen MJ, Zhao TM, Wang H, Wang R, Liu QF, et al. A multicentre studyon the pathogenic agents in 665 adult patients with community-acquired pneumonia in cities of China. Zhonghua Jie He He Hu Za Zhi. 2006 Jan;29(1):3–8.
23. Venkatesan P, Gladman J, Macfarlane JT, Barer D, Berman P, Kinnear W, et al. A hospital study of community acquired pneumonia in the elderly. 1990;45(4):254–258.
24. García-Rey C, Aguilar L, Baquero F, Casal J, Dal-Ré R. Importance of local variations in antibiotic consumption and geographical differences of erythromycin and penicillin resistance in Streptococcus pneumoniae. J Clin Microbiol. 2002 Jan;40(1):159–164.
25. Lynch JP 3rd, Zhanel GG Streptococcus pneumoniae: does antimicrobial resistance matter? Semin Respir Crit Care Med. 2009 Apr;30(2):210–238.
26. Mendell G, John B, Dolin R. In Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases, 7th edition. Philadelphia, PA: Churchill Livingstone; 2009.