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BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF PATHOGENIC ISOLATES OF LOWER RESPIRATORY TRACT FROM MUHIMBILI NATIONAL HOSPITAL, DAR-ES-SALAAM, TANZANIA

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ABSTRACT
This study project was aimed to identify the best antimicrobial agents which will best treat Lower Respiratory Tract Infections. The study was carried out at the Central Pathology Laboratory of the Muhimbili National Hospital, Dar es Salaam. Each sample was inoculated on MacConkey agar, Chocolate agar and Blood agar. The inoculums on the plate were streaked out for discrete colonies with a sterile wire loop. The culture plates were incubated at 37°C for 18-24 hours (overnight) and observed for growth. All the bacteria were processed and the isolates were identified by using standard microbiological techniques and antibiotic sensitivity test was determined by Kirby Bauer disc diffusion method. Results of this study showed that the most common organisms isolated was *Str. Pneumoniae* (45.2%), *Str. Pyogenes* (29.0%), *P. aeruginosa*(12.9%), *K. Pneumoniae* (12.1%) and *H. influenzae*(0.8%). In considering the gram classification, 74.2% of all isolates were gram-positive cocci while 25.8% was gram-negative rod-shaped isolates. The susceptibility patterns varied from one bacterial isolates to the other depending on the drug used. Most of the isolates were susceptible to Vancomycin, Ceftazidime, Penicillin, Gentamicin, Amikacin, Ampicillin, Imipenem, Tobramycin, Ciprofloxacin, Cefotaxime and Chloramphenicol. In conclusion, for an effective management of LRTIs an ultimate and detailed bacteriological diagnosis and susceptibility testing required to overcome global problem of antibiotic resistance.

KEYWORDS: Antibiotic, Lower Respiratory Tract Infections, Susceptibility, Bacteria.

INTRODUCTION
Lower Respiratory Tract (LRT) comprises parts below the larynx namely the bronchi and alveoli of the respiratory system (RS). These parts are mostly affected by infections called the Lower Respiratory Tract Infections (LRTIs). There are three major serious infections affecting lower respiratory tract (LRT) namely, the acute-bronchitis, bronchiolitis and pneumonia. Also, the Lower Respiratory Tract Infections(LRIs) such as sore throat, tuberculosis, whooping cough (Pertussis), emphysema, pharyngitis, ear-ache, laryngitis common cold, otitis media, mastoiditis, sinusitis, tonsillopharyngitis amongst others the most frequently occurred infections of all human diseases and have been documented. LRTI represents one of the most common bacterial diseases encountered in medical practice today, affecting people of all ages from the neonate to the geriatric age group (Kunin, 1994). Inflammation of the lung is a serious condition, responsible for most of the deaths caused by infection of the respiratory tract (TR), especially in adults and infants.

LRTIs are considered as one of the major public health problems and a leading cause of morbidity and mortality in many developing countries it is a global problem accounting for over 50 million deaths of each year and occurs in both community and health care settings (Zafar *et al*., 2008). LRT Infections can arise from other people by cross-infection or even from environmental sources. Respiratory Tract infections are usually contracted through air and by direct contact; this can be by inhalation of small infectious nuclei containing the pathogens, sharing the domestic utensils like cups and other eating materials, kissing and direct inhalation, of pathogens released by infected persons during sneezing, coughing or talking (Mahmoud and Hanan, 2012).

From the epidemiological data taken at KCMC Tanzania during October and November 2013 in assessing the cause of death in children suffering from LRTIs, the most bacteria implicated were *Streptococcus pneumonia* (Ramdani-Bougessa and Rahal, 2003). The most common bacteria implicated as causative agents of Respiratory tract infection are *Pseudomonas* spp., *Streptococcus* spp., *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp., *Enterobacter* spp., *Acinetobacter* spp., and *Haemophilus influenzae*. These bacteria are frequently resistant to commonly used antibiotics such as ampicillin, amoxicillin and kanamycin (Kumari *et al*., 20007). Due to increased resistance of antimicrobial agents of LRTIs, The medical practitioners are obliged to seek several solutions to counteract the problem. One of the solutions required to be taken by the practitioners is the determination of the antibiotic susceptibility patterns of the pathogenic profile of the LRTIs. After the susceptibility has been determined following the administration of the required antibiotics which was susceptible; this problem of antibiotics resistance will be controlled.

This research is designed to identify the best antimicrobial agents which will best treat LRTIs, Identify the alternative antimicrobial agents which will be used in absence of the first line antibiotic, Address the medical field on the clinical antimicrobial agents available in treatments of the LRTIs, Control the microbial resistance obtained from treatment of the LRTIs. The results of this study will be valuable to the medical practitioners for it will assist in administering the sensitive antimicrobial agents to cure LRTIs.

METHODOLOGY

Study Site

The research was held at Central Pathology Laboratory in Muhimbili National Hospital (CPL-MNH) sited in Ilala Municipal, Dar-es-salaam Tanzania.

Study Design

Analytical study which was Hospital based on both prospective and retrospective study covering 124 samples of patients collected from 1st January 2016 to 1st June 2016.

Inclusion and Exclusion Criteria

Inclusion criteria All Pathogenic isolates was included in the study.

Exclusion criteria All Normal flora isolates was eliminated from the study.

Sampling Technique

The sampling technique was systematic sampling from patients admitted at MNH-CPL where by samples admitted was used in the study.

Sample Size Estimation

The sample size was estimated using the minimum sample required to have 90% of pathogenic isolates, 5% level of confident interval of 95%. This was calculated using the formula below

$$N = \frac{Z^2 P (1-P)}{£^2}$$

Where,

N=Maximum sample size
Z=Standard normal deviation **(Z=1.96)**
P=Proportional from literature review.
£=Maximum likely error tolerance.

Sample Collection

The specimens were collected aseptically from 124 (Sputum 106 and Throat swab 18 samples) patients. All patients were instructed on how to collect the sputum samples aseptically and taken to the laboratory immediately for analysis. The sputum samples were collected into well-labeled sterile, wide mouthed glass bottles with screw cap tops. Using a sterile cotton swab, the inner surface of the infected throat was swabbed gently and then the swabs were transported to the laboratory.

Bacteriology

In the laboratory, each sample was inoculated on MacConkey agar, Chocolate agar and Blood agar. The inoculums on the plate were streaked out for discrete colonies with a sterile wire loop. The culture plates were incubated at 37°C for 18— 24 hours (overnight) and observed for growth through the formation of colonies. In incubation the MacConkey and the chocolate agar were incubated aerobically while Blood agar was incubated anaerobically to support fastidious isolates that cannot grow aerobically. All the bacteria were isolated and identified using morphological characteristics, microscopy (gram stain technique) and biochemical tests.

Identification of the Bacterial Isolates

Bacterial isolates were identified by morphology of the colonies and biochemical tests.

Morphology

Streptococcus spp,

***Streptococcus pyogenes*:** They are gram positive, spherical cocci occurring in chains of varying lengths, chain formation is due to the cocci dividing in one plane only and the daughter cells failing to separate completely. In Blood agar the colonies were small (0.5—1mm) in diameter, semitransparent, grey—white with a matt or glossy surface. The colonies were surrounded by a wide zone of beta haemolysis. Some of the colonies were mucoid, it might be due to the strains which produced large capsules. The presence of hyaluronic acid gave the colonies a glistening, watery appearance.

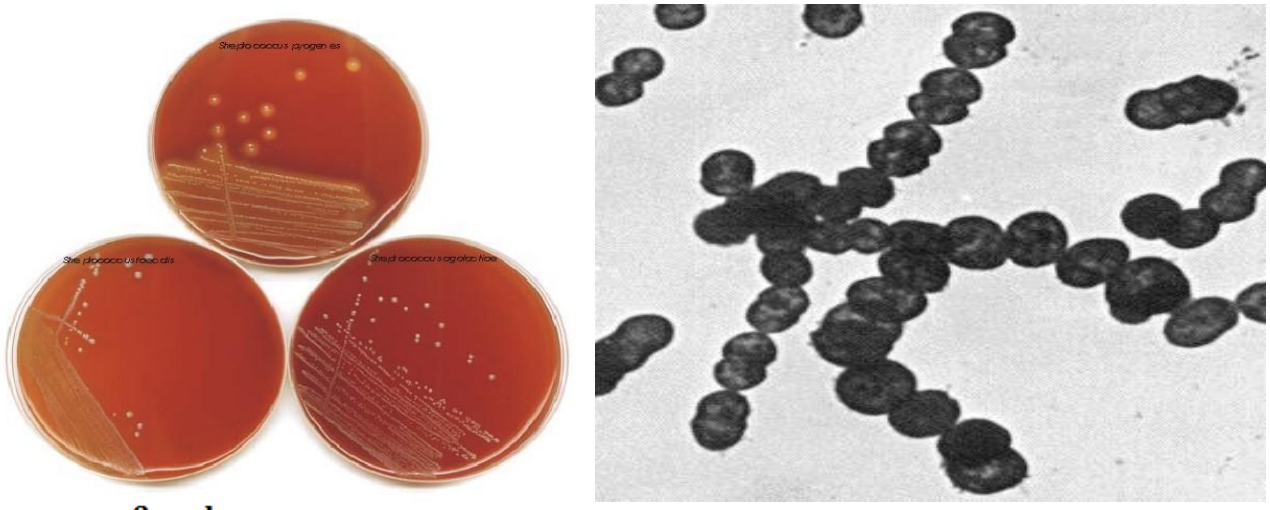


Figure 1: α, β and γ Haemolytic patterns of str. species on blood agar and the gram-positive appearance of the *Strep. pyogenes* microscopically.

Streptococcus pneumonia

They are gram positive cocci, non-motile and non-sporing strains. In the film the isolates showed gram positive cocci arranged in pairs of flame-shaped cocci about 1µm in diameter. On blood ager the colonies were small (0.5—1mm in diameter), moist, mucoid, transparent colonies. Other colonies formed were small, round, translucent colonies, it might be due the strains which were poorly encapsulated. The colonies were surrounded by a 2—3 mm zone of alpha haemolysis. But on farther re-incubation, the colonies on blood agar were surrounded by a zone of beta haemolysiss which might be due to the liberation of oxygen-labile pneumolysin by pneumococci.

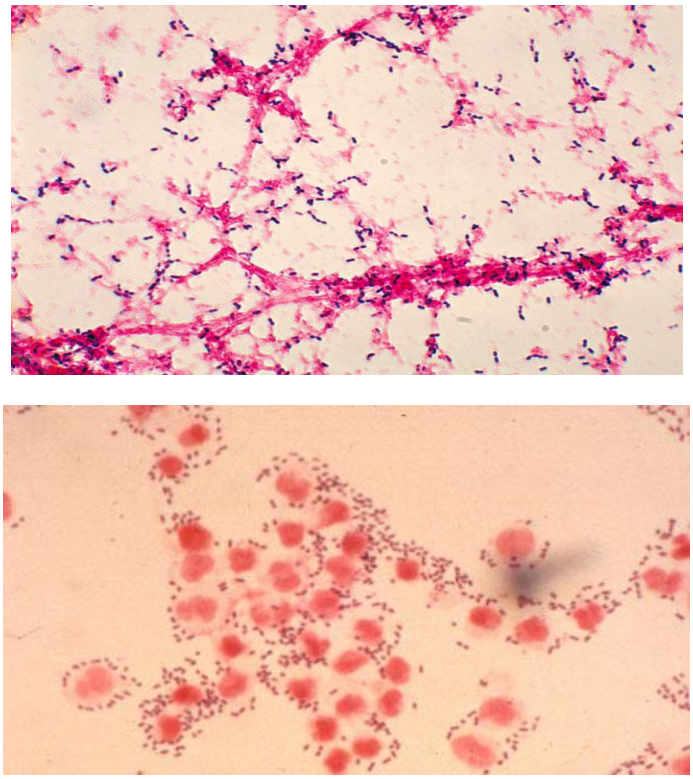


Figure 2: The gram-positive observation of the *Str. pneumoniae* in the film.

Klebsiella pneumonia

The genus *Klebsiella* belongs to the tribe *Klebsielleae*, in the family Enterobacteriaceae. It is a non-motile and non-sporing bacilli. In the film it was observed as gram negative rod-shaped. They are arranged in singly or in pairs. On MacConkey agar the colonies were large, mucoid, raised. Wet, shiny and red in color.

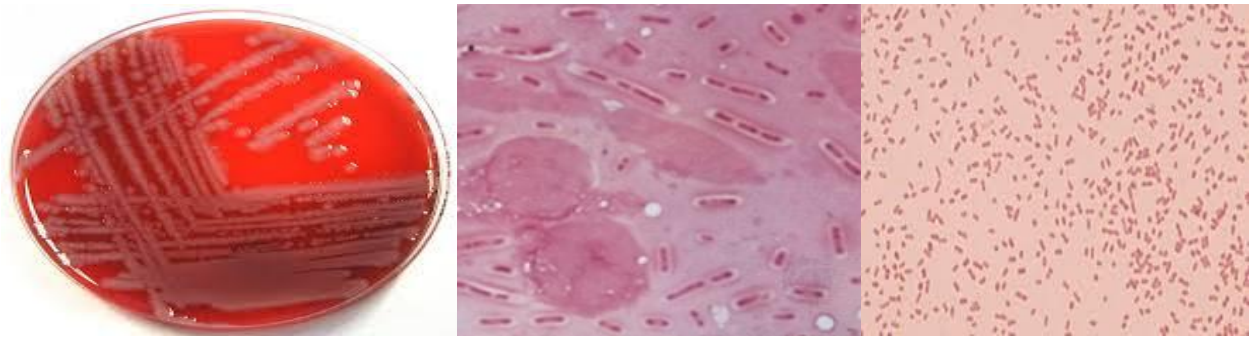


Figure 3: *Kleb. pneumoniae*colonies on blood agar and its film viewed on the microscope.

Pseudomonas aeruginosa

In the film they were gram negative rod-shaped, On MacConkey agar they formed the non-lactose fermenting colonies. On CLED agar the colonies were non-fermented, smooth, large 2—3mm in diameter, round, translucent, irregularly, with a fluorescent greenish colour, On blood agar most strains were haemolytic. Also on both type of media the growth had the characteristics of emitting a fruity odour (grape—like odour).

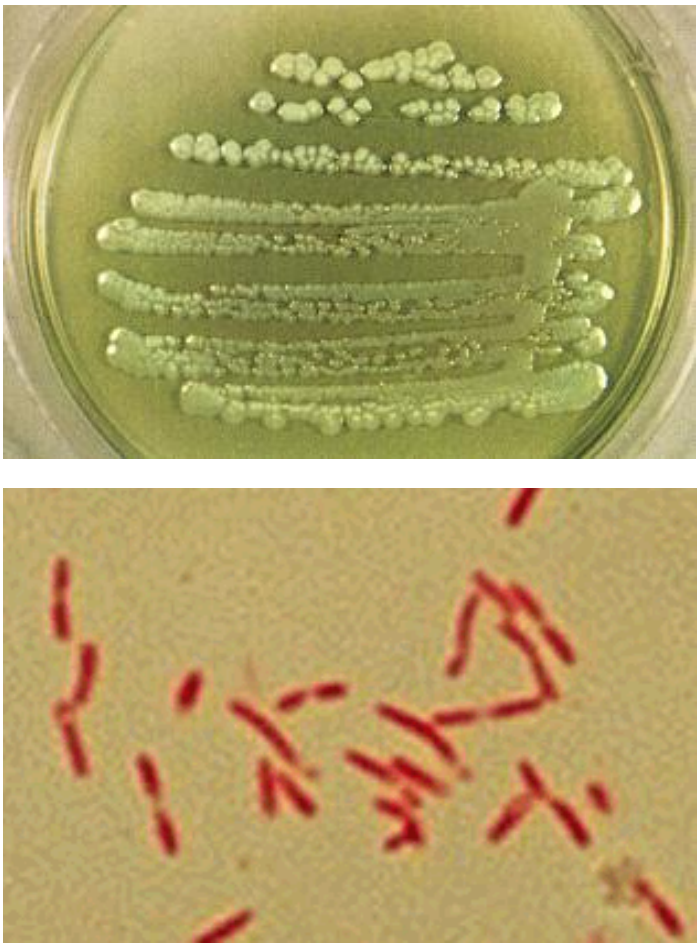


Figure 4: *Pseud. aeruginosa* colonies on CLED agar and the sketch of gram negative film of the *pseud. aeruginosa.*

Haemophilus influenza

On the blood film these were gram negative rod or cocco bacillus. On the culture plate they had the fastidious growth requirements, among the requirements they require growth factors V and X which are only found in blood agar, which is essential for its growth. In this case literally, this strain of *H. influenzae* forms the **satellitism** when they are streaked with *S. aureaus* overnight. It is because the *S. aureaus* streak provide X factor, and *Staphylococcal cells* themselves secrete V factor during logarithmic growth. On blood agar and Chocolate agar the colonies were large, round, grey and opaque.

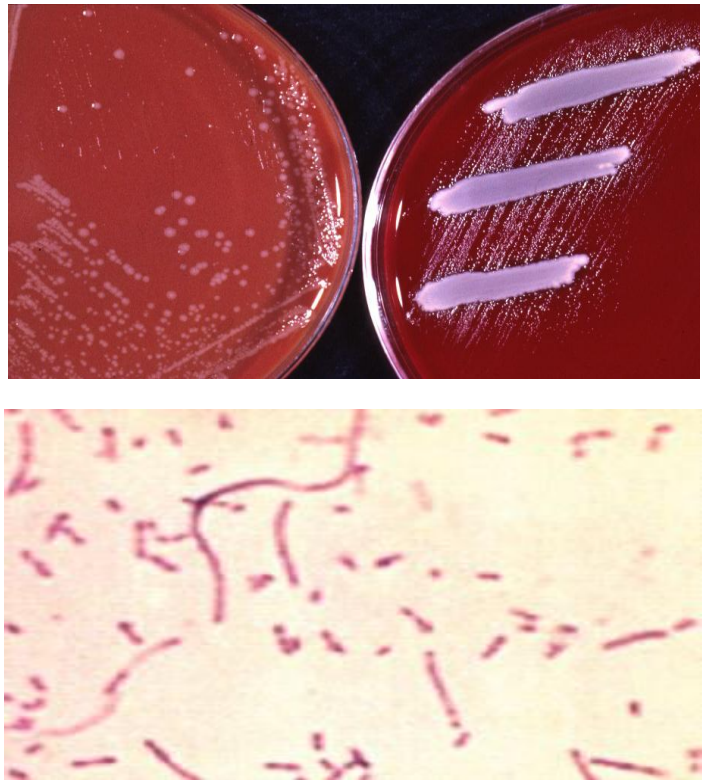


Figure 5: BAP showing satellitism and the gram negative film from the the plate.

Biochemical tests

The biochemical tests which were used in identification were, catalase, oxidase, indole, urease Citrate utilization, MR, VP, (IMViC), β—Bacitracin disk and α—optochin disk.

Table 1: The biochemical tests.

Biochemical tests	Pseud. aeruginosa	Kleb. pneumoniae	H. influenzae	Strep. pneumoniae	Strep. pyogenes
Catalase	+		+	−	−
Oxidase	+	−	+		
Indole	−	−	+		
Urease	−	+	+		
Lactose fermentation	−				
β—Bacitracin disk sensitivity				−	+
α—optochin disk sensitivity				+	−

Antibiotics Sensitivity Testing (ast).

The antimicrobial sensitivity of the test strains of twenty four antibacterial drugs was done using the Kirby-Bauer disk diffusion method. The commercial available antibiotic discs used for the study were vancomycin (VA) 30µg/mL, ceftazidime (CAZ) 30µg/mL, penicilinG (P) 10µg/mL, gentamicin (CN) 10µg/mL, ampicilin (AMP) 10µg/mL, clindamycin (DA) 2µg/mL, tetracycline (TE) 30µg/mL, trimethoprim–sulfamethoxazole (SXT) 1.25/23.75, erythromycin (E) 15µg/mL, chloramphenical(C) 30µg/mL, cephalothin (KF) 30µg/mL, ceftriaxone (CRO) 30µg/mL, Test for ciprofloxacin (CIP) 5µg/mL, imipenem (IMI) 10µg/mL, azithromycin (AZM) 15µg/mL, meropenem (MER) 10µg/mL, cephalexin (CL) 30µg/mL, amikacin (AK) 30µg/mL, cefotaxime (CTX) 30µg/mL, tobramycin (TOB) 10µg/mL, nitrofurantoin (F) 300µg/mL and novobiocin (NO) 30µg/mL. A lawn of test pathogen was prepared by evenly spreading 100µl inoculums with the help of a sterilized spreader onto the entire surface of the agar plate using the culture suspension adjusted to 0.5 McFarland's standards. The plates were allowed to dry before applying antibiotic disc. Then, some commercially available antibiotic discs were gently and firmly placed on the agar plates, which were then left at room temperature for 1 h 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 24hours using a scale. An organism was interpreted as highly susceptible, Intermediate and Resistant according to the manufacturer guidelines.

Data Analysis

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) program while other parameters like antibiotic susceptibility pattern and antibiotic resistance was calculated using the ordinary chi(x^2) technique.

3.8: Ethical Consideration

Ethical consideration permission to conduct the study was sought from IMTU Research committee.

RESULSTS

From January to June 2016 more than 150 samples were processed in the bacteriology laboratory at MNH-CPL, out of which 124 isolates were included in the study. Samples which showed no growth (NBG) were excluded from the study. Out of the included samples 18 samples were from throat swab and 106 samples from sputum.

Table 2: Number and percentage of Sputum and Throat Swab.

Specimen type	No. of samples	Percentage
Sputum	106	85.5%
Throat swab	18	14.5%
Total	124 specimens	100%

Table 3 shows the percentage of GPC and GNR. Results of the study showed that the most common organisms isolated was 45.2% *Str. Pneumoniae*, 29.0% *Str. Pyogenes*, 12.9% *P. aeruginosa*, 12.1% *K. Pneumoniae* and 0.8% were *H. influenzae*. In considering the gram classification, 92 out of the bacterial isolates were gram-positive cocci constituting 74.2% while 32 which are 25.8% were gram-negative rod-shaped isolates.

Table 3: Percentage of GPC and GNR.

Isolates	% of the isolates
Gram positive cocci	74.2%
Gram negative rod	25.8%

The susceptibility of the clinical isolates to routinely used antibiotics in Muhimbili National Hospital is presented in the table 4. *Str.pneumoniae* was the most prevalent isolate among all bacteria with susceptibility of 64.3% vancomycin and 39.3% penicillin G. *Str.pyogenes* was the second prevalent bacteria with susceptibility of 63.9% vancomycin and 41.75% penicillin G. *P. aeruginosa* had a susceptibility of 75% amikacin and 62.5% ceftazidime. The fourth prevalent isolate was *K. pneumoniae* with susceptibility of 53.3% imipenem and 40% gentamicin. *H. influenzae* was the least prevalent bacteria which was only one isolate with susceptibility to ceftazidime, imipenem, azithromycin and tobramycin for 100%. However, this might give false susceptible results because of the single isolate obtained.

Table 4: The susceptibility of isolates to commonly used antibiotics in Muhimbili National Hospital from patients of LRTIs from January 1st to June 2016.

Antibiograms	*P.aeruginosa*	*K. pneumoniae*	*H. influenzae*	*Str. peumoniae*	*Str. pyogenes*
Vancomycin (VA) 30µg/mL	—	—	—	64.3%	63.9%
Ceftazidime (CAZ)30µg/mL	62.5%	26.6%	100%	37.5%	36.1%
Penicillin G (P) 10µg/ml	0%	25%	—	39.3%	41.7%
Gentamicin (CN) 10µg/mL	56.25%	40%	—	23.2%	11,1%
Ampicilin (AMP)10µg/mL	6.25%	13.3%	—	30.4%	22.2%
Clindamycin (DA) 2µg/mL	—	0%	—	14.3%	30.6%
Tetracycline (TE) 30µg/mL	—	20%	—	10.7%	8.3%
Trimethoprim–Sulfamethoxazole (SXT) 1.25/23.75	6.25%	13,3%	0%	12.5%	8.3%
Erythromycin (E) 15µg/mL	0%	20%	—	17.9%	19.4%
Chloramphenical (C) 30µg/mL	6.25%	26.7%	—	23.3%	36.1%
Cephalothin (KF) 30µg/mL	6.25%	6.7%	—	21.4%	13.9%
Ceftriaxone (CRO) 30µg/mL	12.5%	33.3%	—	17.8%	13.9%
Ciprofloxacin (CIP) 5µg/mL	43.75%	40%	0%	17.2%	16.7%
Imipenem (IMI) 10µg/mL	25%	53.3%	100%	14.3%	27.8%
Azithromycin (AZM) 15µg/mL	6.25%	26.7%	100%	16.1%	16.7%
Meropenem (MER) 10µg/mL	18.75%	20%	—	12.5%	5.6%
Cephalexin (CL) 30µg/mL	6.25%	13.3%	—	8.9%	11.1%
Amikacin (AK) 30µg/mL	75%	20%	—	12.5%	19.4%
Cefotaxime (CTX) 30µg/mL	43.75%	13.3%	—	7.1%	2.8%
Tobramycin (TOB) 10µg/mL	25%	6.7%	100%	**7.1%**	8.3%
Nitrofurantoin (F) 300µg/mL	—	0%	—	3.6%	11.1%

DISCUSSION

Among the specimens processed, From sputum 106 (85.5%) samples and throat swab 18 (14.5%) revealed that *Str.pneumoniae* was the most prevalent isolate with 45.1% of all isolates while *H. influenzae* showing the least prevalence among all isolates with 0.8%. This agreed with the research conducted in Tamilanadu, India, between June to December 2012 (Manikandan and Amsath, 2013). where the most prevalent gram positive cocci from all isolates observed were *Str.pneumoniae.* Also, *Streptococcus pneumonia* was more susceptible to most of the beta lactam antibiotics of the class cephalosporins, penicillins and carbapenems and also it was susceptible to aminoglycosides. The susceptibility observed shows that the susceptibility was below 50%. Similarly, *Str. pyogenes* showed similar susceptibility to *Str. pneumoniae* although the values are different, but *K.pneumoniae* was highly susceptible to beta lactam antibiotics of the class carbapenems including imipenem with 53.3% and aminoglycosides including Gentamicin with 40% susceptibility. Most values in this study showed the susceptibility below 50%. This might be because of the decrease in sensitivity of the antibiotics commonly used clinically to the increase in drug resistance to most strains of the Lower Respiratory Tract Pathogens (WHO, 2012).

Hancock (1998) reported that *P. aeruginosa* shows high resistant from carbapenem group of antibiotics, quinolones and third generation cephalosporin. In this study, the susceptibility of amikacin to *P. aeruginosa* is 75%. This is similar to the findings of Rotimi *et al*. 1998 which reported 68% amikacin susceptibility to *P. aeruginosa*. Generally, *P. aeruginosa* have been detected to be more resistant to most antibiotics which are commonly used (Quinn, 1998). The predominance of *P. aeruginosa* resistance is considered as a serious problem in many countries (Agarwal *et al*., 2006). There was a single *H. influenzae* in this study and its susceptibility percentages gives a 100% to each sensitive antibiotics. *H. influenzae* is a fastidious bacteria which require factor V and factor X for its growth and this is the one which form satellitism when streaked with *S. aureus.*

The prevalence of different phenotypes of microorganisms in sputum and throat swab of patients with respiratory illness should be closely monitored. A great challenges comes from the physicians in developing countries who treat LRTIs symptomatically as a result the pathogens affecting the patients develop changes due to insensitive antibiotics prescribed which is then mutate to resistant strains for most drugs treating LRTIs. Therefore, they should take samples of the Sputum and throat swab for Culture and sensitivity to the laboratory before prescribing any antimicrobial agent to avoid and decrease the multi-drug resistance among the pathogenic isolates of LRTIs.

In conclusion, for an effective management of lower respiratory tract infection an ultimate and detailed bacteriological diagnosis and susceptibility testing required to overcome global problem of antibiotic resistance and by encouraging greater understanding of this problem different solutions can be planned by health care providers.

REFERENCES

1. Kumari, H.B.V., Nagarathna.S., and Chandramuki, A. 2007. Antimicrobial resistance pattern among aerobic gram negative bacilli of lower respiratory tract specimens of intensive care unit patients in a neurocentre. Indian. *J. Chest. Dis. Allied. Sci.,* 49: 19-22. Org. 86, 408-416.
2. Mahmoud Aly and Hanan H Balkhy. 2012. The prevalence of antimicrobial resistance in clinical isolates from Gulf Corporation Council countries. *Antimicrobial Resistanceand Infection Control*, 1: 26 doi 10.1186/2047-2994-1-26.
3. Zafar, A., Hussain, Z., Lomama, E., Sibiie, S., Irfan, S., Khan, E. 2008. Antibiotic susceptibility of pathogens isolated from patients with community-acquired respiratory tract infections in Pakistan- the active study. *J. Ayub Med. Coll. Abbottabad.,* 20(1): 7-9.
4. WHO, 2012. Antimicrobial resistance: no action today, no cure tomorrow, World Health Da - 7 April.
5. Manikandan, C. and Amsath, A. 2013. Antibiotic susceptibility of bacterial strains isolated from patients with respiratory tract infections. *Int. J. Pure Appl. Zool.,* 1(1): 61-69.
6. Kunin CM (1994). Urinary trat infections in female. Clin infect, Dis., 18: 1-12.
7. Ramdani-Bougessa N, Rahal K. Serotype distribution and antimicrobial resistance of *Streptococcus pneumonia* isolated in Algiers, Algeria. *Antimicrob Agents Chemother,* 2003; 47: 824-6.
8. Hancock, R.E.W (1998) Resistance mechanism in pseudomonas aeruginosa and other nonfermentative gram-negative bacteria. *Clin Infect Dis.,* 27: 289–299.
9. Quinn, JP 1998) Clinical problems posed by multi resistant on fermenting gram negative pathogens. *Clin Infect Dis.,* 7: 117–124.
10. Rotimi, V.O., Al-Sweih, N.A., Feteih, J. (1998). The prevalence and antibiotic susceptibility pattern of gram-Negative bacterial isolates in two ICUs in Saudi Arabia and Kuwait. *Diagn Microbiol Infect Dis.,* 30: 53–59.
11. Agarwal, R., Guptha, D. and Ray, P. 2006. Epidemiology, risk factors and outcome of nosocomial infections in a respiratory intensive care unit in north India. *J. infect.,* 53(2): 98-105.