ISSN Print/Online: 2320-9577/2320-9585
INTERNATIONAL JOURNAL OF PURE AND APPLIED ZOOLOGY
Volume 1, Issue 1, January-March 2013
Available online at: http://www.ijpaz.com

RESEARCH ARTICLE

ANTIBIOTIC SUSCEPTIBILITY OF BACTERIAL STRAINS ISOLATED FROM PATIENTS WITH RESPIRATORY TRACT INFECTIONS

C. MANIKANDAN* AND A. AMSATH
P.G. and Research Department of Zoology, Khadir Mohideen College,
Adirampattinam-614701, Tamil Nadu, India

* Corresponding Author E-mail: drmanikandan66@gmail.com, Tel: +91 9361299995

Article Info: Received on: 15.02.2013, Accepted on: 05.03.2013 Published online: 15.03.2013

ABSTRACT

This study was aimed to observe the susceptibility pattern of bacterial isolates from respiratory tract infection (RTI). The study was carried out between June, 2012 and December, 2012. Sputum and throat swab specimens were collected aseptically from patients and cultured on the appropriate bacteriological media. Bacterial isolates were identified by biochemical tests and antimicrobial susceptibility performed by standard methods. Out of 360 specimens, some 337 (93.6%) species of various bacteria isolated. The prevalence of bacteria spp. isolated were as follows *Streptococcus pneumoniae* (36%), *Klebsiella pneumoniae* (28.4%), *Staphylococcus aureus* (24%), Pseudomonas *aeruginosa* (11%) and *Escherichia coli* (0.6%). The susceptibility patterns varied from one bacterial isolates to the other depending on the drug. The susceptibility test against microbial isolates of 13 commercially used antibiotics was used. Most of the isolates were susceptible to Amikacin, Cefotaxime, Ofloxacin, Netilmycin, Ciprofloxacin and Ceftriazone. 50 % of the bacterial spp. was resistant to the other antibiotics. Most of the bacteria were shown 92.6% sensitivity to Amikacin. Pseudomonas aeruginosa was 99% resistant to ampicillin, amoxicillin, 98% resistant to roxithromycin, 96% resistant to sparfloxacin and cefuroxime. These findings have clinical and epidemiological significance.

Keywords: Respiratory infection, sputum, throat swab, bacterial strains, antibiotic susceptibility.

INTRODUCTON

Respiratory tract infection (RTI) is considered as one of the major public health problems and a leading cause of morbidity and mortality in many developing countries (Bipin Prajapati *et al.*, 2011; Jacobs *et al.*, 2009 and Sharma *et al.,* 2005). 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). Infection can arise from other people by cross-infection or even from an environmental sources. The microorganisms larger than 10µm usually are trapped by the hair and cilia lining the nasal cavity. Coughing and sneezing reflexes clear the respiratory system of microorganisms by expelling them forcefully from the lungs through the mouth and nose respectively (Prescott *et al.*, 2005).

The most infections are limited to the upper respiratory tract and only 5% involve the lower respiratory tract, respectively. Upper Respiratory infections (URTIs) involve the nasal passages, pharynx, tonsils and epiglottis (Veloo *et al.,* 2012). The nasal discharge associated with colds contains virus particles, dead cells from the nasal mucosa and bacteria. Lower respiratory tract infections (LRTIs) involve the bronchi and alveoli (Anderson *et al.,* 1993). They include two serious conditions–acute

bronchitis and pneumonia. Inflammation of the lung is a serious condition, responsible for most of the deaths caused by infection of the respiratory tract, especially in adults and infants.

The most common bacteria implicated as causative agents of Respiratory tract infection was included *Pseudomonas* spp., *Streptococcus* spp., *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp., *Enterobacter* spp., *Acinetobacter* spp., and *Haemophilus influenza* (Riley and Riley, 2003; Rudan *et al.*, 2008). These bacteria are frequently resistant to commonly used antibiotics such as ampicillin, amoxicillin and kanamycin (Kumari *et al.*, 2007).

Antimicrobial resistance developed by microbes against antibiotics open serious debates in this issue and recognized as a serious problem by global medicinal and research community (Finch, 2004; Kumari *et al.*, 2007:). Many factors play in the emergence of resistance (WHO, 2012) from poor utilization of antimicrobial agents, to the transmission of resistant bacteria from patient to patient and from healthcare workers to patients and vise versa, to a lack of guidelines for a appropriate and judicious use of antimicrobial agents, to lack of easy-to-use auditing tools for restriction (Mahmoud Aly and Hanan H Balkhy. 2012). In addition, there is a clear misuse of antimicrobial in the animal industry, those are the same agents used in humans. All these factors together led to the inevitable rise and emergence of resistance. This study was, therefore, conducted to determine the antibacterial susceptibility of bacteria isolated from respiratory tract infections.patients in Pattukkottai area, Tamil Nadu state, India.

MATERIALS AND METHODS

Study population: In the present study, a total of 360 clinical samples were collected from patients who attended the various hospitals and clinical wards in Pattukkottai, Tamilnadu State, India. All patients had clinical evidence of respiratory tract infections, as determined by the treating doctors. Only a single positive culture per patient was included in the analysis.

Specimen collection: The specimens were collected aseptically from 360 (Sputum 248 and Throat swab 112 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. For a collection of throat specimens, the handle of a spoon was used to depress the tongue to examine the mouth for the presence of inflamed membrane, exudates or pus. The study was carried out for six months between July 2012 - Dec 2012.

Bacteriology: In the laboratory, each sample was inoculated on McConkey agar, Chocolate agar and Blood agar. The inoculum on the plate was streaked out for discrete colonies with a sterile wire 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 Sharma (2008).

Antibiotic susceptibility testing: The antimicrobial sensitivity of the test strains of thirteen antibacterial drugs was done using the Kirby-Bauer disk diffusion method (Bauer *et al.*, 1966). The commercial available antibiotic discs used for the study were Amikacin (AK) (30µg), Amoxacillin (AX) (10µg), Ampicillin (AM) (10µg), Cefotaxime (CF) (30µg), Ceftriaxone (CT) (30µg), Cefuroxime (CE) (30µg), Ciprofloxacin (CP) (05µg), Erythromycin (ER) (15µg), Gentamycin (GM) (10µg), Netilmycin (NT) (30µg), Ofloxacin (OF) (05µg), Roxithromycin (RX) (30µg) and Sparfloxacin (SF) (5µg). A lawn of test pathogen (1ml of an 18 hours peptone broth culture) was prepared by evenly spreading 100µl inoculums with the help of a sterilized spreader onto the entire surface of the agar plate. 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 24 hours using a scale. An organism was interpreted as highly susceptible if the diameter of inhibition zone was more than 19 mm, intermediate if diameter was 15-18 mm and resistant if the diameter was less than 13 mm. The intermediate readings were considered as sensitive in the assessment of the data.

RESULTS

A total of 360 patients were screened for having RTIs, out of which, a total number of 337 (93.6%) were included in the study. Of the samples 23 (9.3%) showed no growth and other samples analyzed 337 species of various bacteria were isolated. This consisted of 225 (90.7%) from sputum and 112 (33.2%) from throat swab as detailed Table 1.

Table 1. Number of samples collected from Sputum and Throat Swab.

Specimens	No. of samples	Growth observed and %
Sputum	248	225 (90.7%)
Throat swab	112	112 (100%)
Total	360	337 (93.6%)

The most common organism isolated was *Streptococcus pneumoniae* (36%), *Klebsiella pneumoniae (*28.4%), *Staphylococcus aureus* (24%), *Pseudomonas aeruginosa* (11%) and *Escherichia coli* (0.6%) (Table 2). The Gram Positive cocci constituted 202 (60 %) while Gram Negative Bacilli constituted 135 (40 %) of the total isolates (Table 3).

The susceptibility of the clinical isolates to use routinely prescribed antibiotics in hospital Table 4. *S. pneumoniae was* the most prevalent bacteria with a susceptibility of 98 % Amikacin, 88% Cefotaxime, 83% Netilmycin, 82% Ciprofloxacin, 77% Ceftriaxone, 66.3% Amoxicillin, 66.3 Ofloxacin, 59.6% Erythromycin, 56% Sparfloxacin, 55% Cefuroxime, 55% Roxithromycin, 39.3% Gentamycin and 28% Ampicillin. The susceptibility profile of *S. aureus* 97%Amikacin, 91% Cefotaxime, 83% Ciprofloxacin, 80% Netilmycin, 77% Ceftriaxone, 73% Cefuroxime, 68% Sparfloxacin 67.2% Amoxicillin, 64.5%, Ofloxacin, 60% Roxithromycin, 38.5% Erythromycin, 32% Gentamycin and 21.3% Ampicillin.

Table 2. Bacterial species detected from sputum and throat swab of patients with RTI.

Causative Pathogens	No	Percentage
Streptococcus pneumoniae	121	36.0 %
Klebsiella pneumoniae	96	28.4 %
Staphylococcus aureus	81	24.0 %
Pseudomonas aeruginosa	37	11.0 %
Escherichia coli	02	0.6 %

Table 3. Percentage of GPC and GNB.

S. No.	Pathogens	% of organisms
1	Gram Negative Bacilli	40%
2	Gram Positive Cocci	60%

K. pneumoniae was the second most prevalent bacteria with a susceptibility of 95% Amikacin, 65.4% Amoxicillin, 56% Ciprofloxacin, 53.5% Ofloxacin, 52.7% Erythromycin, 50% Netilmycin, 42.3% Ampicillin, 40% Cefotaxime, 38.8% Gentamycin, 35% Ceftriaxone, 15% Sparfloxacin, 10% Cefuroxime, and 10% Roxithromycin. The susceptibility profile of *E.coli* 86% Amikacin, 64.6% Ofloxacin, 63% Ceftriaxone, 54% Ciprofloxacin , 51% Netilmycin, 48% Cefotaxime, 47.5% Cefuroxime, 43.7%, Erythromycin, 20% Amoxicillin, 20% Gentamycin, 15.7% Ampicillin, 15% Roxithromycin and 14% Sparfloxacin.

P. aeruginosa had a susceptibility profile of 87% Amikacin, 47% Cefotaxime, 43% Ceftriaxone, 38% Netilmycin, 21% Ofloxacin, 20.5% Gentamycin, 20% Ciprofloxacin, 08% Erythromycin, 04% Cefuroxime, 04% Sparfloxacin, 02% Roxithromycin, 01% Amoxicillin, and 01% Ampicillin.

Table 4: Susceptibility of the isolates to commonly used antibiotics.

Antibiotics	*S. pneumoniae*	*S. aureus*	*K. pneumoniae*	*P. aeruginosa*	*E.coli*
Amikacin	98	97	95	87	86
Amoxicillin	66.3	67.2	65.4	01	20
Ampicillin	28	21.3	42.3	01	15.7
Cefotaxime	88	91	40	47	48
Ceftriaxone	77	77	35	43	63
Cefuroxime	55	73	10	04	47.5
Ciprofloxacin	82	83	56	20	54
Erythromycin	59.6	38.5	52.7	08	43.7
Gentamycin	39.3	32	38.8	20.5	20
Netilmycin	83	80	50	38	51
Ofloxacin	66.3	64.5	53.5	21	64.6
Roxithromycin	55	60	10	02	15
Sparfloxacin	56	68	15	04	14

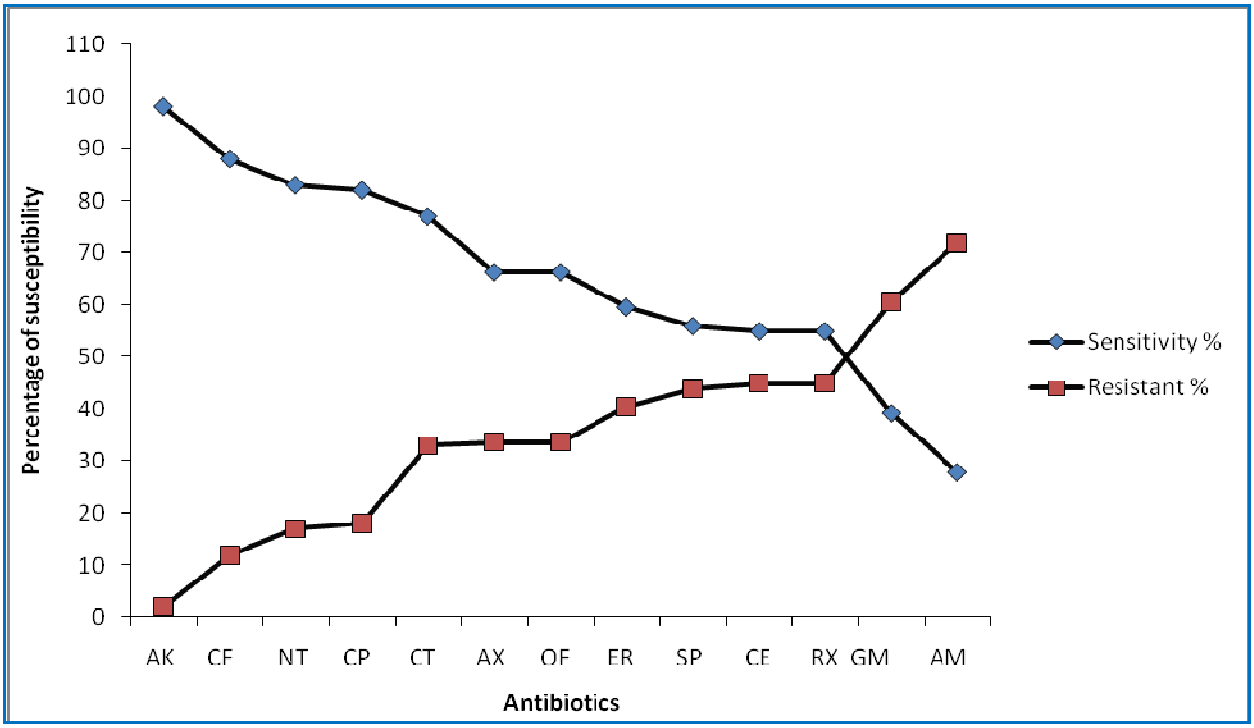

Figure 1. Susceptibility patterns of *S.pneumoniae*.

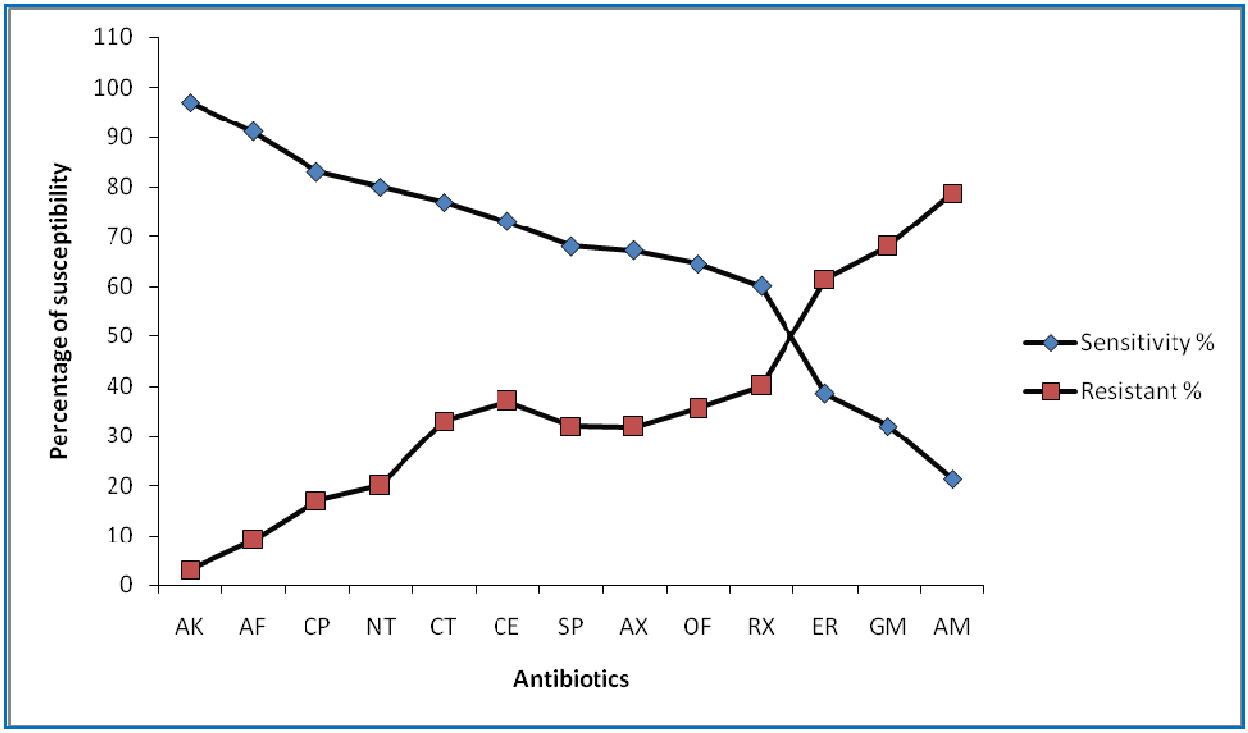

Figure 2. Susceptibility patterns of *S.aureus.*

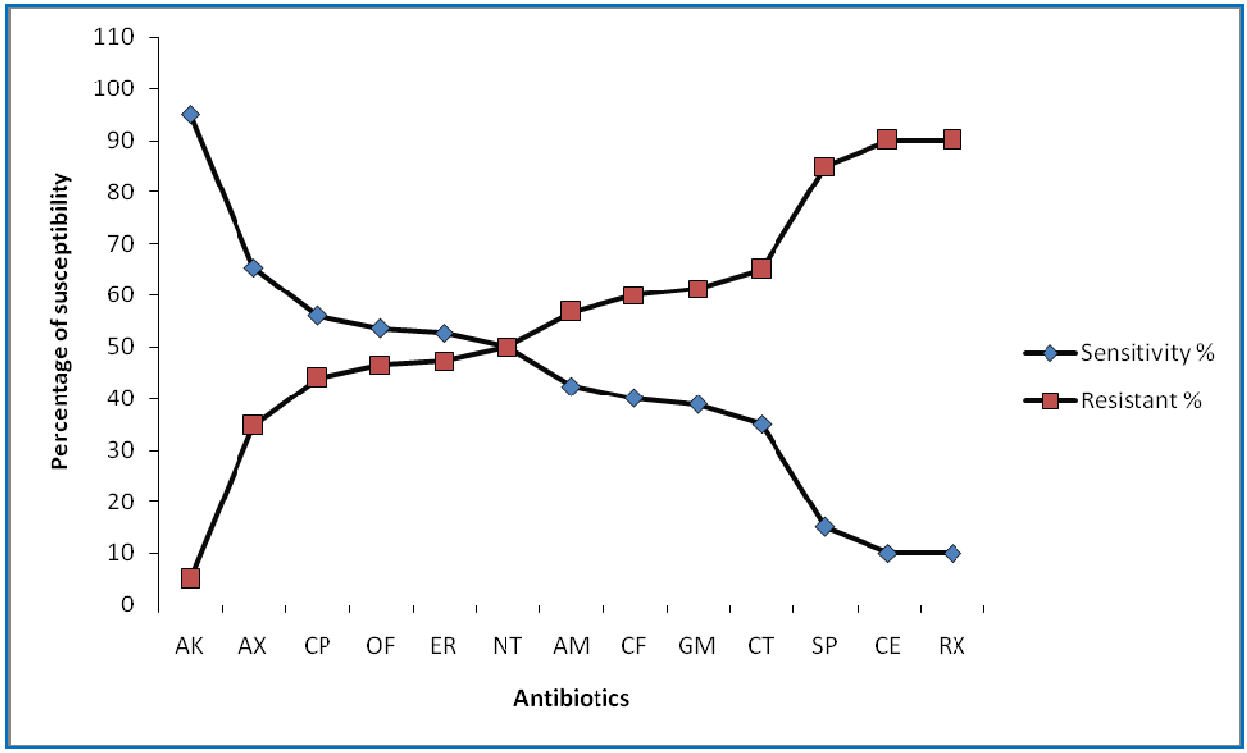

Figure 3. Susceptibility patterns of *K.pneumoniae.*

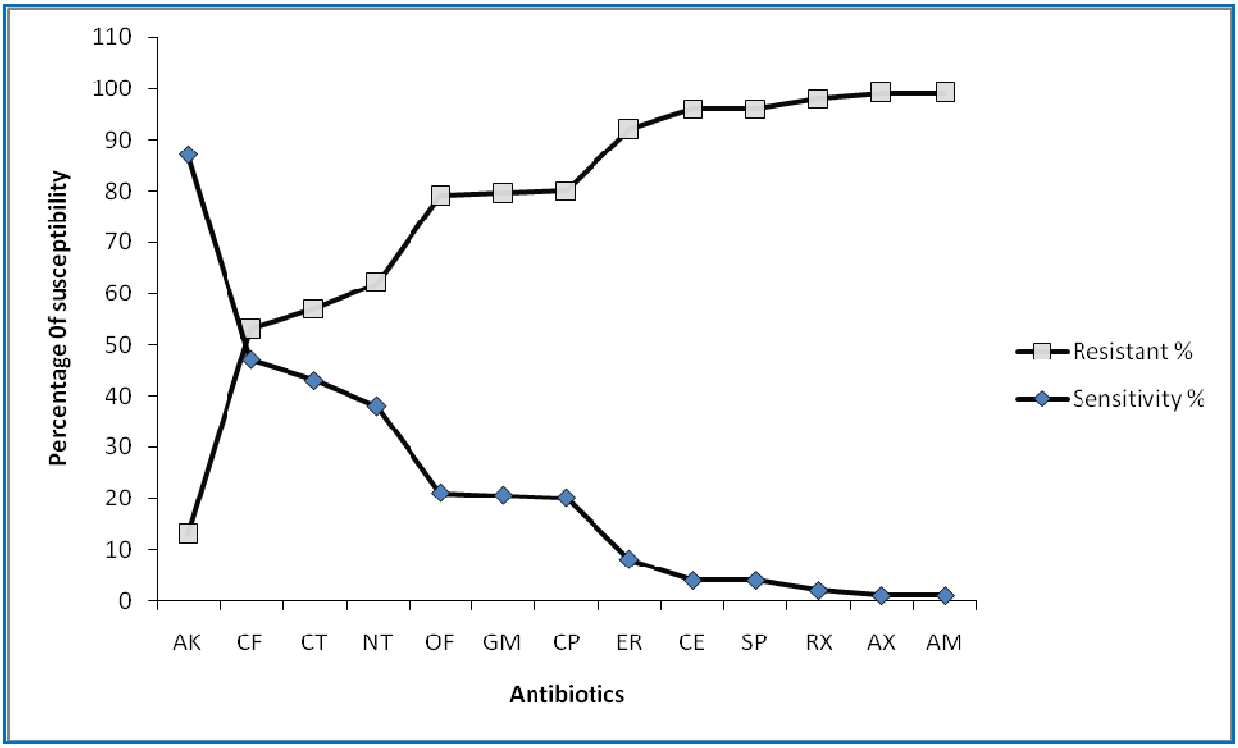

Figure 4. Susceptibility patterns of *P.aeruginosa*.

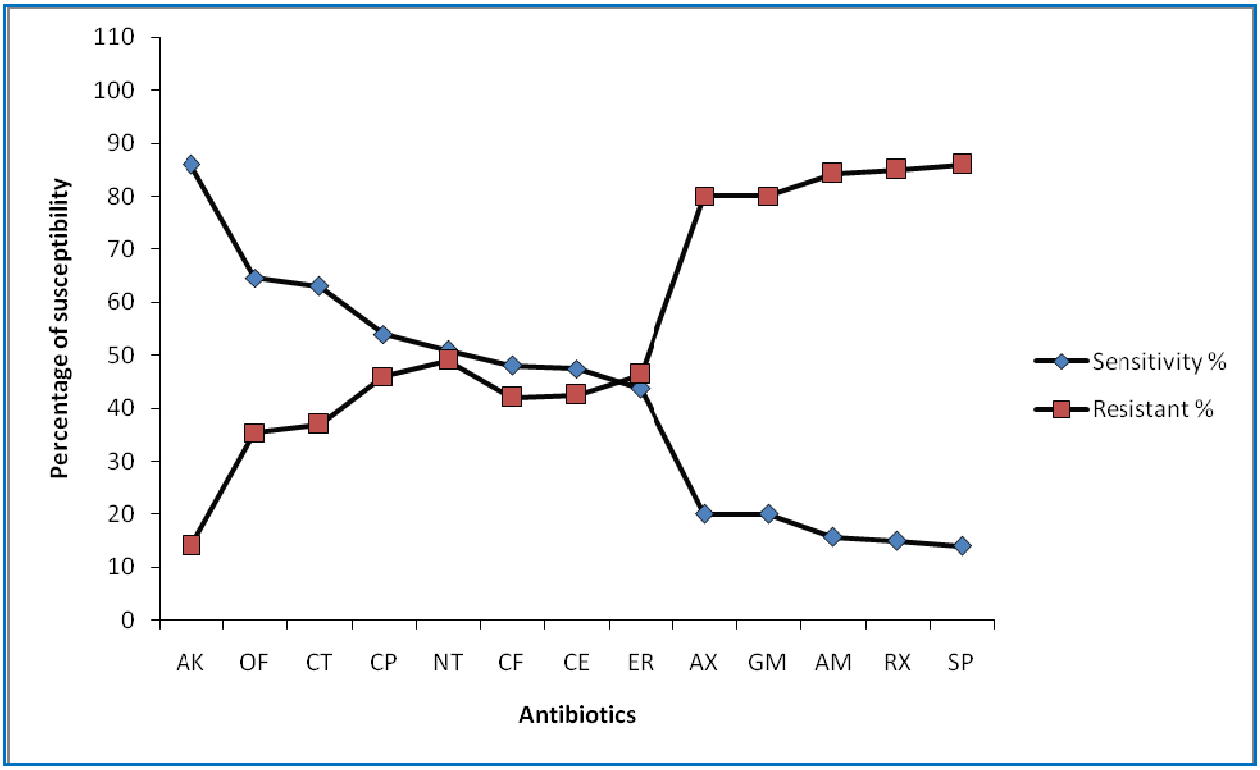

Figure 5. Susceptibility patterns of *E.coli*.

DISCUSSION

A total of 360 patients were for having RTIs, out of which a total number of 337 were included in the study. The bacteria isolated from the samples included *S. pneumoniae, K pneumoniae*, *S aureus, P. aeruginosa* and *E.coli.* These isolates clearly represented clinically significant pathogens were similar to the results obtained in India (Kumari *et al.,*2007) and also falls within the range of frequencies reported in other countries such as Nigeria (El-Mohamed *et al*., 2010; Okesola and Oni, 2009), Turkey (Ezgi Ozilmaz *et al.,* 2005).

All the isolates displayed variable sensitivity to the antibiotics tested as detailed as shown in table 4. *S. pneumoniae* showed a more sensitivity pattern of 98% (AK), 88% (CF), 83% (NT), 82% (CP), 77% (CT), 66.6% (AX, OF), 59.6% (ER) and other antibiotics were showed susceptibility to below 50%. Similar pattern obtained from other studies (Okesola and Ige, 2008). The incidence of isolation of ampicillin-resistant strains of *S. pneumoniae* in the present study was as high as that in intensive medication-oriented clinics in Japan (Naoyuki Harada *et al.,* 2013) and USA (Watanabe *et al.,* 1995). The resistance rate of S. *pneumoniae* to 40.4% (ER), 33.7% (OF, AX), 33% (CT), 18% (CP), 17% (NT), 12% (CF), and 2% (AK). *S. aureus* had a similar susceptibility profile that of *S. pneumoniae*.

The majority of the *K. pneumonia* were sensitive to 95% (AK), 65.4 (AX), 56% (CP), 53.5 (OF), 52.7 (ER), 50% (NT) and other antibiotics showed susceptibility to below 50%. It shows a gradual increase in resistance and a decrease in sensitivity (Sarathbabu *et al*., 2012). In the present study, only two numbers of *E. coli* (0.6%) were isolated and it had a similar susceptibility profile that of K. *pneumoniae* (Amin *et al.,* 2009, Akpan *et al.,* 2011).

All the *P. aeruginosa* were susceptible to 87% (AK), 47% (CF), 43% (CT) and other strains were shown very low susceptibility. The lower susceptibility compared very well to that of other studied conducted in other countries (Anab Fatima *et al.,* 2012). The predominance of *P. aeruginosa* resistance considered as a serious problem in many countries (Agarval *et al*., 2006). The reason of 11% *P aeruginosa* prevalence in our study is unclear. The possibility of patients who acquired RTIs and also have coexisting chronic lung disease such as bronchiectasis and destructive lung due to previous pulmonary tuberculosis. As it is known that *P. aeruginosa* commonly colonies and some time cause overt infection to the destructive lungs (Komus *et al*., 2006). In our study the rate of amikacin resistance was found to be relatively high i.e., 13% in a previous hospital study resistance rate among *Pseudomonas aeruginosa* was only 5-9% against amikacin (Bouza *et al*., 1999).

Conclusion

The most of the isolates had a high level of resistance to examine antibiotics. The reason for the resistance may be due to indiscriminate use and abuse of drugs, adulteration of drugs and mutation of microorganisms. This problem indicates the importance of performing antibiotic susceptibility testing before empirical therapy. Health sectors should educate public on proper usage of antimicrobial agents.

ACKNOWLEDGEMENTS

The authors would like to thank the Principal and HOD of Zoology, Khadir Mohideen College, Adirampattinam-614701 for the facilities provided to carry out the work and the Senior Laboratory Technologists of Gangasaras Diagnostic and Research Centre, Pattukkottai-614601, Thanjavur district, Tamilnadu, India for their consistent support and help in this study.

REFERENCES

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

Akpan, M. M., Itah, A. Y., Akinjogunla, O. J., and Eshiet, U. M. 2011. Antibiotic Susceptibility Profile of Bacteria spp. Isolated from Patients with Recurrent Cough in Cross River State, Nigeria. *Arc.App.Sci.Res.,* **3**(4):179-185

Amin, R., Hoque A. M. W., Khan R. F. and Rahman M. 2009. Considering respiratory tract infections and antimicrobial sensitivity: An

exploratory analysis. *Malaysian J. Microbiol.,* **5**(2): 109-112.

Anderson, H., Esmail, A., Hollowell, J., Littlejohns, P., and Strachen D. 1993. Epidemiologically based needs assessment: lower respiratory disease. DHA Project Research Prograrnme; pp 6-12.

Bauer, AW., Kirby, WMM., Sherris, JC., and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*., **45**: 493-496.

Bipin Prajapati., Nitiben Talsania., Sonaliya, K. N. (2011). A study on prevalence of acute respiratory Tract infections (ARI) in under five children in Urban and rural communities of Ahmedabad District, Gujarat, *Nat. J. Com. Med.,* **2**(2): 255-259.

Bouza, E., Garcia-Gorrote, F., Cercenado, E., Martin, M., and Diaz, M.S. 1999. *Pseudomonas aeruginosa*: a survey of resistance in 136 hospitals in spain. , 43(4):981.

El-Mahmood, A.M., Isa, A., and Tirmidhi, S.B. 2010. Antimicrobial susceptibility of some respiratory tract pathogens to commonly used antibiotics at the Specialist Hospital, Yola, Adamawa State, Nigeria. *J. Clin. Med. Res.* **2**(8), pp. 135-142.

Ezgi Ozyilmaz, Ozay Arikan Akan, Meral Gulhan, and Kamruddin Ahmed. 2005. Major bacteria of community acquired respiratory tract infection in Turkey. Jpn. *J. Infect. Dis*., **58**:50-52.

Finch, R.G. 2004. Antibiotic resistance: a view from the prescriber. *Nat. Rev. Microbiol.,* **2**(12):989-994.

Jacobs, E., Dalhoff, A., and Korfmann, G. 2009. Susceptibility patterns of bacterial isolates from hospitalised patients with respiratory tract infections. *Int. J. Antimicrob. Agents,*. **33**: 52–57.

Komus, N., Tertemiz, KC., Akkolu, A., and Gulay, Yilmaz, E. 2006. *Pseudomonas aeruginosa* colonization in brochoiectatic patients and clinical reflections. *Tuberk Toraks.* **54**:355-362.

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.

Mahmoud Aly and Hanan H Balkhy. 2012. The prevalence of antimicrobial resistance in clinical isolates from Gulf Corporation Council countries. *Antimicrobial Resistance and Infection Control*, **1**:26 doi: 10.1186/2047-2994-1-26

Naoyuki Harada., Noriko Hasegawa., Hidenori Seki., Yuko Okua., Masayuki Totsuka., Takashi Hatori and Jun Murakami. 2013. Severe Pneumonia Caused by *Streptococcus Pneumoniae* and *Haemophilus Influenzae* Co infection in an Elderly Patient: A Case Report Nobuhiro Akuzawa. Elmer press, J Med Cases**4**(1):4-8

Okesola, A.O., and Ige, O.M. 2008 Trends in Bacterial Pathogens of Lower Respiratory Tract Infections, *Indian J Chest Dis Allied Sci* ; **50**: 269-272

Okesola, AO., and Oni, AA. 2009. Antimicrobial resistance Among Common Bacterial Pathogens in South Western Nigeria. Am-Eurosian, *J. Environ. Sci.,* **5**(3): 327-330.

Prescott, L. M., Harley, J. R., and Klein, D. A. 2005. Microbiology (6th Ed.) McGraw-Hill. Higher Education, Boston Burride, pp 673-936.

Riley, C., and Riley, S. 2003. Influenza and pneumococcal disease in the community. Nursing Standard **18**(4): 45–51.

Rudan, I., Boschi-Pinto, C., Biloglav, Z., Mulholland, K. and Campbell, H. 2008. Epidemiology and etiology of childhood pneumonia. *Bulletin of the World Health.* org **86**: 408-416

Sarathbabu, R., Ramani, T.V., Bhaskara rao, K., and Supriya Panda. 2012. Antibiotic

susceptibility pattern of *Klebsiella pneumoniae* isolated from sputum, urine and pus samples. *J. Pharmacy and Bio. Sci.* ISSN: 2278-3008 **1**: 04-09.

Sharma, K., 2008. Manual of Microbiology: Tools and Techniques, [2nd Ed.] Ann Books Gopaljee Enterprises, Dehi, India. pp. 163-165; 181-7.

Sharma, R., Sharma, C. L., and Kapoor, B. 2005. Antibacterial resistance: current problems and possible solutions. *Indian Journal of Medical Sciences* **59**, 120-129.

Veloo, AC., Seme, K., Raangs, E., Rurenga, P., Singadji, Z., Wekema-Mulder, G., and van Winkelhoff, AJ. 2012. Antibiotic susceptibility profiles of oral pathogens. *Int J Antimicrob Agents.* Nov; **40**(5):450-4.

Watanabe, A., Oizumi, K., Matsuno, K., Nishino, T., Motomiya, M., and Nukiwa, T. 1995. Antibiotic susceptibility of the sputum pathogens and throat swab pathogens isolated from the patients undergoing treatment in twenty-one private clinics in Japan. *Tohoku J Exp Med.* Apr; **175**(4):235-47.

WHO, 2012. Antimicrobial resistance: no action today, no cure tomorrow, World Health Day - 7 April.

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.

Cite this article as:
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.