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A prospective cross-sectional study on assessment of antibiotic susceptibility pattern in patients with respiratory tract infections in Nilgiris district

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ABSTRACT

Respiratory tract infections (RTIs) are one of the most common infectious diseases globally imposing a significant increase in morbidity and mortality in the developing countries. There is an exponential increase in antibiotic resistance attributed to indiscriminate use of antibiotics, lack of monitoring of antibiotic susceptibility patterns, cross infections, etc. The study was carried out to isolate and identify the common bacteria causing RTIs among the patients attending two secondary care hospitals. Sputum and throat swab samples were collected from clinically diagnosed RTI in 50 patients, in an aseptic condition and then cultured on the appropriate bacteriological media. Antimicrobial susceptibility testing was performed by Kirby–Bauer disk diffusion method and results were interpreted according to the Clinical Laboratory Standards guidelines. The antibiotic susceptibility testing revealed Amikacin, Gentamicin, and Ceftriaxone as highly sensitive and ciprofloxacin a widely used antibiotic in Nilgiris as the most resistant one.

INTRODUCTION

Respiratory tract infections (RTIs) are one of the most common infectious diseases globally imposing a significant increase in morbidity and mortality in the developing countries. Most of the infections affect the upper respiratory tract and merely 5% constitute the lower RTIs (LRTIs). However, LRTIs are more serious and debilitating than upper RTIs (URTIs) (Shanmugam *et al*., 2015; Sharma *et al*., 2015; Taura *et al*., 2013). URTIs are common in individuals of all the age group and include Pharyngitis, Nasopharyngitis, Tonsillitis, Otitis Media, and Sinusitis. These infections are primarily caused by viral assault, secondary infection with bacteria, such as *Haemophilus influenzae*, *Streptococcus pyogenes*, *Moraxella catarrhalis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*, can complicate the illness (Kousalya *et al*., 2010; Wang *et al*., 2016). The LRTIs mainly affect bronchi and alveoli causing bronchitis and pneumonia. Gram positive bacteria such as *Staphylococcus aureus*, *Streptococcus pneumonia* and gram negative organisms such as *H. influenzae*, *Pseudomonas* species, *Klebsiella* species, Acinetobacter are the major culprits of LRTIs. Factors such as age, gender, season, smoking, immunosuppressive states link to the causatives of RTIs (Khan *et al*., 2014; Kumar *et al*., 2015).

There is an exponential increase in antibiotic resistance attributed to indiscriminate use of antibiotics, lack of monitoring of antibiotic susceptibility patterns, and cross infections. In India, most hospitals follow empirical therapy, which fails to work upon the emergence of resistance and adds additional cost to the patient and the healthcare sector. Therefore, it is of utmost importance to assess the antibiotic susceptibility pattern of local bacterial species which aids in developing an antibiotic policy. The aim of this study is to determine the antibiotic susceptibility pattern of bacterial isolates in patients with RTIs in our region.

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MATERIALS AND METHODS

The study was conducted at Pediatrics, Intensive Care Unit, Female, Male medical ward and Outpatient department of two secondary care hospitals in the area. The study is an Open Label, Prospective, and Cross-sectional study. The Pediatrics and adult population of age 6–65 years old with a clear diagnosis of RTI were included in our study. Patients who have been taking antibiotics for infections other than respiratory infections and patients who are not willing to provide the informed consent were excluded from the study.

The Institutional Review Board approval was obtained (approval no: JSSCP/DPP/IRB/03/2017-18, approval date: 03/02/2018) before initiation of the study. Sputum and throat swab samples were collected from clinically diagnosed RTI in 50 patients by simple random sampling after obtaining the informed consent and the patient data were entered using the patient profile form. Out of the 50 samples collected by purposive sampling, 35 samples were throat swab and 15 were sputum samples. Sputum and Throat swab samples were collected using their respective standard procedures. Proper labeling was done and the samples collected were brought to the Microbiology lab within 20 minutes and inoculated in the nutrient broth. Following which they were incubated at 35°C ± 2°C for 24 hours for observation of bacterial growth (Fig. 1) (Balaji, 2015; Sharma, 2008). Out of the 50 samples, 41 were found to be positive of bacterial growth and nine samples showed no growth. The selective Media used were Cetrimide agar medium, Mannitol salt agar medium, Mac Conkey agar medium , and Blood agar medium (Fig. 2) (Balaji, 2015; Gazi *et al*., 2004; Kousalya *et al*., 2015). These Media were selected because of their ability to facilitate the growth of *P. aeruginosa*, *S. aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *S. pneumoniae*, which are the causative organism for most RTIs. The Media were then incubated at 35°C ± 2°C for 24 hours to isolate the desired bacterial species. The identified organisms were tested for Antibiotic susceptibility using Kirby–Bauer Disc Diffusion method as per the standards of Clinical Laboratory Standards Guidelines (CLSI) (Balaji, 2015; Hudzicki, 2009).

Figure 1. Inoculation and incubation of samples in nutrient broth.

The readymade antibiotic discs were selected based on the commonly used antibiotics to treat RTIs in hospitals in Ooty. To increase precision, similar concentrations were used. The panel of antibiotic discs chosen for the isolated organisms includes Amikacin (AK 30 µg), Gentamicin (HLG 120 µg), Cefotaxime (CTX 30 µg), Ceftriaxone (CTR 30 µg), Azithromycin (AZM 15 µg), and Ciprofloxacin (CIP 1 µg) (Balaji, 2015; Hudzicki, 2009).

The Zone of inhibition was measured using a metal calliper and the results were interpreted according to the standard reference CLSI (Fig. 3). The results were then reported as sensitive, intermediate, and resistant according to the criteria set by CLSI. Descriptive statistics were used to present the distribution of samples, the growth of bacteria, gender, and age distribution (Balaji, 2015).

RESULTS

In our study, we collected about 50 samples, out of which 35 (70%) throat swab samples are collected from the patients with URTI and 15 (30%) sputum samples are collected from the patients with LRTI.

From the collected samples, 41 (82%) showed growth and 9 (18%) samples did not show any growth. Figure 4 shows equal gender distribution among male and female.

The highest predominant age group with RTI is patients between the age group 19 and 35 years as depicted in Figure 5.

Around three samples were collected in the month of January, 38 samples in the month of February and nine samples during March. Out of the male patients, 17 (34%) were smokers and all female patients, including 33 pediatric patients (66%), were non-smokers.

About 45 organisms were isolated in our study, among which 36 (80%) are Gram-positive and 9 (20%) Gram-negative organisms.

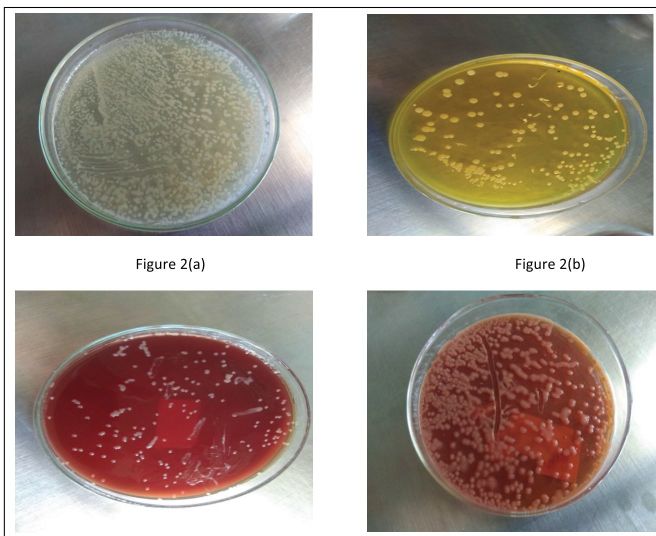

Figure 2(a) Figure 2(b)

Figure 2(c) Figure 2(d)

Figure 2. Isolation of bacterial species. (a) Isolation of *P. aeruginosa* on Cetrimide agar. (b) Isolation of *S. aureus* on Mannitol Salt Agar medium. (c) Isolation of *K. pneumoniae* on blood agar. (d) Isolation of *K. pneumoniae* on MacConkey agar

The overall distribution of isolated bacterial species in our study is shown in Figure 6.

Antibiotic susceptibility testing

The most common causative organism found in our study is *S. aureus* and the sensitivity pattern of various antibiotics are as follows: AK (94.12%) is the most sensitive, followed by HLG (88%), AZM (82%), CTR (58.82%), CTX (47%), and the highly resistant antibiotic is CIP (53%). Isolated *E. coli* is sensitive to HLG (60%) followed by CIP (40%), AK (20%) and highly resistant to CTX (100%) followed by CTR (80%). *Pseudomonas aeuroginosa* is highly sensitive to AK (100%), HLG (100%), followed by CTR (50%), and resistance pattern observed in them were as follows; CIP (50%) and CTX (50%). *Klebsiella pneumoniae* had intermediate susceptibility to CTX (100%) and CTR (50%) is sensitive to this organism (Table 1). Even if *Staphylococcus epidermidis* has 38% occurrence, it is of no significance in our study as it is not a causative organism of RTI, but it is sensitive to almost all the antibiotics used in our study.

DISCUSSION

RTIs are one of the most common and the biggest threats to global health, which can affect anyone of any age in any country. It includes both upper and lower RTIs among which URTI is most common. Even though LRTIs contribute to a lesser percent of infections they are potentially fatal (Sharma *et al*., 2015; Taura *et al*., 2013). The rapid emergence of antibiotic resistant bacteria is making the antibiotic therapy ineffective and prolonging the hospital stay thereby causing economic burden and fatality in the patient. Our study, focus mainly on collecting data on the risk factors associated with RTIs, the prevalence of the causative organism responsible for RTIs and observing their susceptibility pattern to various antibiotics, such as AK, HLG, CIP, AZM, CTX, and CTR. During the 6 months of the study period, 50 samples were collected from respiratory tract infected patients, out of which, 70% were swab (URTIs) and 30% were sputum samples (LRTIs), this is contrary to a study conducted by Manikandan and Amsath (2013) study where the pattern was vice versa. This shows a marked difference in the distribution of URTI and LRTI. From the collected samples, 41 had growth, which is in accordance with sample distribution in Taura *et al*. (2013) study. The distribution of samples among male patients was 42%, female patients were 46%, and 12% in pediatrics is in accordance with El-Mahmood *et al*. (2010) and is opposing Taura *et al*. (2013). The prevalence of RTI in various age groups were as follows: 52% in 19–35 years, 22% in 50–65 years, 14% in 36–49 years, and 12% in 5–18 years, this similar pattern is seen in a study conducted by Taura *et al*. (2013). The highest prevalence of RTI in young and middle age group can be due to the high exposure of these age groups to the risk factors, such as smoking, alcoholism, and work exposure. All the samples were collected in the autumn season.

From the 41 positive samples, 45 bacteria were isolated, out of which 80% was Gram-positive and 20% were Gram-negative which is slightly similar to the result observed by Manikandan and Amsath (2013) study. The overall prevalence of isolated bacterial species was 38% *S. aureus*, 11% *E. coli*, 9% *P. aeruginosa*, and 4% *K. pneumoniae*. In LRTI, the highest pathogenic bacterium is *S. aureus*, which is dissimilar to the results obtained in studies conducted by Khan *et al*. (2015), Saxena *et al*. (2015), and Srivasthava *et al*. (2013). The same bacterial species were isolated by Bajpai *et al*. (2013) and Manikandan and Amsath (2013) in their study conducted on LRTI samples. Other than these isolated organisms, 38% *S. epidermidis* was obtained in our study, which is not a causative organism of RTI, and, therefore, is not considered in our study. This can be attributed to the patient suffering from other infections which were not ruled out. In URTI, the most common organism is *S. aureus* followed by *P. aeuroginosa* and *S. epidermidis*, a similar result was detected by Kousalya *et al*. (2010).

Antimicrobial sensitivity pattern of *S. aureus* observed in our study is as follows: AK is the highest sensitive antibiotic which is similar to the sensitivity pattern observed in Manikandan and Amsath (2013) study, followed by AZM, CTR, and CTX, whereas in study conducted by Kousalya *et al*. (2010), HLG was highly sensitive to this organism and it was highly resistant to CIP. In our study, *E. coli* is highly resistant to CTX and CTR. It is sensitive to HLG and CIP. *Pseudomonas aeuroginosa* is highly sensitive to AK and HLG followed by CTR; it is found to be resistant to CIP and CTX. Our study is in line with the reports of Wang *et al*. (2015), *K. pneumoniae* had intermediate sensitivity to CTX. CTR was sensitive to this organism, which is in accordance with Bajpai *et al*. (2013) study.

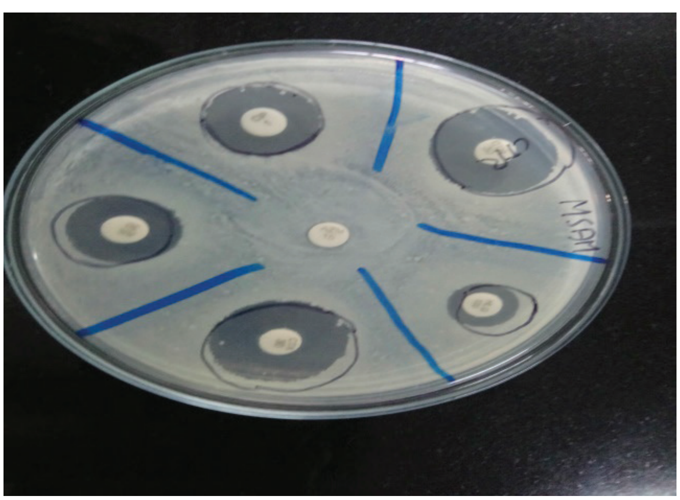

Figure 3. Zone of inhibition by Kirby Bauer disc diffusion method.

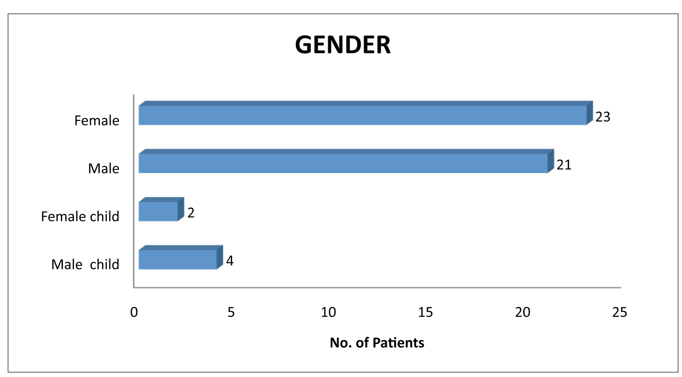

Figure 4. Gender distribution.

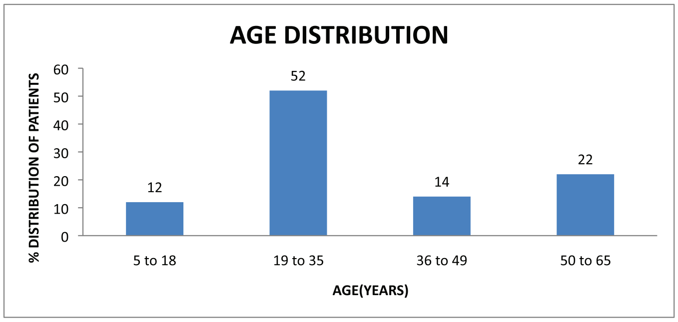

Figure 5. Age distribution of patients.

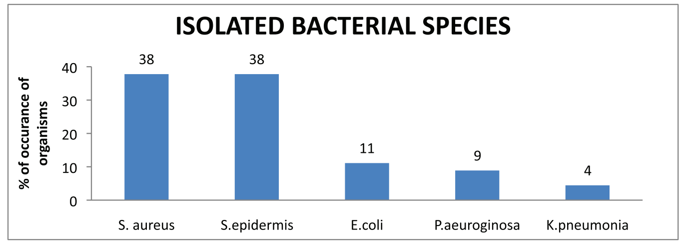

Figure 6. Isolated bacterial species.

Table 1. Antibiotic susceptibility (%).

Antibiotics/organism	*S. aureus* (%)			*P. aereuginosa* (%)			*K. pneumoniae* (%)			*E. coli* (%)			*S. epidermidis* (%)		
	S	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*
AK	94	-	6	100	-	-	N/A	N/A	N/A	20	80	-	100	-	-
HLG	88	-	12	100	-	-	N/A	N/A	N/A	60	40	-	94	6	-
CIP	29	18	53	25	25	50	N/A	N/A	N/A	40	20	40	53	12	35
AZM	82	0	18	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	82	-	18
CTX	47	47	6	25	25	50	-	100	-	-	-	100	35	53	12
CTR	59	29	12	50	-	50	50	50	-	0	20	80	35	53	12

From our study, AK, HLG, and CTR were highly sensitive to almost all the organisms although other third-generation cephalosporin and AZM had intermediate sensitivity pattern (Yadav *et al*., 2014). CIP is resistant against most of the organisms. This susceptibility pattern observed in our study highlights the irrational use of these antibiotics as empirical therapy for RTIs in most of the clinical settings in Ooty, Nilgiris District.

CONCLUSION

From our study, we got *S. aureus* as the most prevalent bacteria responsible for infections. Our study shows that AK, HLG, and CTR were highly sensitive to most of the organisms which are in concordance with the most widely used antibiotics in hospitals in Nilgiris. However, CIP was found to be the highly resistant antibiotic in our study which is surprisingly not the case in practical settings. A high level of morbidity was seen in the majority of patients in our study.

RECOMMENDATIONS AND FUTURE OUTLOOK

Existing management strategies seems insufficient for the control of RTIs as many of the hospitals lack antibiotic susceptibility testing facilities and the treatment is purely empirical. This leads to a huge level of discomfort for both the public and the government. Regional differences in prevalence and resistance patterns of bacteria make it mandatory to establish the treatment guidelines based on antibiotic susceptibility testing.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest in the study.

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None.

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