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ANTIMICROBIAL RESISTANCE OF BACTERIAL AGENTS OF THE UPPER RESPIRATORY TRACT IN SOUTH INDIAN POPULATION

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ABSTRACT

The study was aimed at determining bacterial agents of the upper respiratory tract and the susceptibility patterns of isolates to antibiotics. The throat swab samples from 250 patients suspected of upper respiratory tract infection (URTI) were obtained from the General Medicine outpatient department of a Rural Health Centre of Rajah Muthiah Medical College and Hospital (RMMC and H), Annamalai University, Chidambaram, Tamilnadu, India and inoculated in the culture medium. The bacterial infection was confirmed only in 228 patients. The organisms isolated on medium were identified by their cultural, morphological and biochemical characteristics. *Staphylococcus aureus* was identified as the most prevalent bacterial isolate (45.61%) followed by β *hemolytic streptococci* (22.81%). Thirty four strains (14.91%) were identified as *Klebsiella penumoniae*, 19 (8.33%) as *Pseudomonas aeruginosa* and the rest belonged to α *hemolytic streptococci*, *Escherichia coli* and *Haemophilus influenzae*. All *Staphylococcus* spp. were resistant to penicillin, ampicillin and co-trimoxazole. All the isolates were resistant to at least one antibiotic. The overall resistance rates were generally low for gentamicin, cefixime and ceftazidime respectively.

Key words: antibiotic, susceptible, resistant, acute respiratory infection, bacteria.

INTRODUCTION

Respiratory tract infection is one of the most important infectious diseases worldwide. This infection is the leading cause of morbidity and mortality in critically ill patients in developing

countries [1, 2, 3]. Respiratory tract infections (RTIs), which involve the upper or lower respiratory tract, frequently occurs after birth [4]. RTIs, such as sore thoat, earache, laryngitis, common cold, otitis media, sinusitis, and mastoiditis, are the most frequently-occurred infections of all human diseases and have been frequently documented [4, 5]. Since these infections often seem trivial, they are more commonly discounted as being mere temporary inconveniences that cause transient discomfort [6]. Respiratory infections in particular those occur in upper respiratory tract are seen with great frequency in both children and adults and have remarkable economic impact, related not only to lost output in the workplace but also to the frequent prescription by physicians of antibiotics, even when the causative agents of infection almost certainly are not bacteria [7]. On the other side, respiratory tract infections are the most common reason for primary care consultations [8]. Eradication of the causative agents of respiratory tract infections is recognized as a requirement [9], however during the last few years, the increase in the rates of antibiotic resistance amongst the major microbial causes of the respiratory infections in the community has compromised the selection of empirical treatment for some respiratory tract infections [10]. It has been reported that pneumonia due to secondary bacterial infections is the most important complication of the lower respiratory tract and that surveillance of acute RTIs in defined populations to monitor prevailing pathogens and to determine population groups at special risk are important for taking preventive measures [11, 12]. The main bacterial pathogens recovered from complicated influenza virus infections include *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae, Klebsiella pneumoniae*, and *Streptococcus pyogenes* [13, 14].

To our knowledge, there is limited information on the prevalence of various bacterial pathogens and their antibiotic resistance patterns in Tamilnadu, India. Hence, the aim of this study was to determine the prevalence and antimicrobial susceptibility rates of bacterial strains isolated from thoat specimens obtained from a Rural Health Centre of Rajah Muthiah Medical College and Hospital (RMMC and H), Annamalai University, Chidambaram, Tamilnadu, India.

MATERIALS AND METHODS

Study design and subjects

The field practice area of the Rural Health Centre of Rajah Muthiah Medical College and Hospital (RMMC and H), Annamalai University, Chidambaram,

forms the study area. The Rural Health Centre is located at South Pichavaram, 12 kms to the east of the town of Chidambaram and 4-6 kms from the sea coast (Bay of Bengal). The villages covered by the service area are: 1) South Pichavaram, 2) Kanagarapattu, 3) T.S. Pettai, 4) North Pichavaram, 5) Keezhaperambai and 6) Senjicherry. These villages form part of the Chidambaram Taluk in the Cuddalore district of the state of Tamil Nadu, South India.

Subjects coming to the General Medicine outpatient department were included in the study. In total, 250 patients took part in the study, of which the bacterial infection was confirmed only in 228 patients. Out of 228 patients, 108 were males and 120 were females. The study subjects were aged 10-60 years with a mean age of 35 years. All the subjects were informed of the aim of the study and were assured that all information supplied by them will remain confidential. Their verbal consent and cooperation to participate in the study was then solicited. Ethical approval was obtained before carrying out the study. The criteria for inclusion in the study were: the patients must be suffering from URTIs, referred to clinics or hospitals, and must not have been taking antibiotics of any kind at least for the two weeks preceding the study. The subjects presented URTIs, including sore thoat, otitis media, runny nose, sinusitis and cough. The large majority (78%) of them had runny nose and sore thoat while sinusitis was the least noted. The symptoms lasted for 3-14 days.

BACTERIAL ISOLATES

The consulting physicians themselves collected 250 throat swabs. The specimens were transported to the Microbiology Laboratory of the Department of Microbiology, Annamalai University, Chidambaram for analysis. The specimens were collected in nine months. A loopful of each sample was inoculated onto blood agar, chocolate agar, and eosin methylene blue agar. The inoculated plates were incubated at 37°C for 24-48 h aerobically, except for chocolate agar, in which the plates were incubated for 24-48 h at 37°C in an atmosphere of 5-10% CO_2 [15]. After incubation, macroscopic and microscopic examinations of colonies on plates were carried out, and suspect colonies were subcultured on appropriate solid culture media for purification. They were later sub cultured on appropriate slants and stored at 4°C for further analysis. Pure cultures were presumptively identified based on their cultural and morphological characteristics on selective and differential media [15]. Standard microbiological techniques

and biochemical tests were employed to confirm isolates.

Antimicrobial susceptibility testing

The Kirby-Bauer disc-diffusion test was used as previously described [16]. A sterile cotton swab was dipped into the standardized solution of bacterial cultures and used for evenly inoculating Mueller-Hinton plates (Himedia, Mumbai) and allowed to dry. Thereafter, antibiotic discs with the following drug contents-ampicillin (10 µg), ceftriaxone (5 µg), chloramphenicol (10 µg), co-trimoxazole (25 µg), erythromycin (10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), cefixime (5 µg), ceftazidime (30 µg) and penicillin (10 µg) were placed on the plates, spacing them well to prevent the overlapping of inhibition zones. These antibiotics were selected based on prescription practices for URTIs in our locality. The plates were incubated at 37°C for 24 h, and the diameters were measured. The results were read as recommended by the NCCLS [17].

RESULTS AND DISCUSSION

A total of 228 throat specimens had positive results for bacterial cultures. One hundred and four isolates (45.61%) were identified as *S. aureus*, 52 (22.81%) as *β hemolytic streptococci*, 34 (14.91%) as *Klebsiella pneumoniae*, 19 (8.33%) as *Pseudomonas aeruginosa*, 11 (4.83%) as α *hemolytic streptococci*, 5 (2.91%) as *E. coli* and 3 (1.32%) as *H. influenzae*. Of the 228 isolates identified, 53% were recovered from females compared to 47% from males. The susceptibility patterns of isolates to various antibiotics are shown in Table 1. *S. aureus* was resistant to penicillin, ampicillin and co-trimoxazole. The lowest susceptibility was observed for all the isolates to penicillin. Gentamicin and cefixime generally exhibited good activity against the isolates. All the isolates were resistant to atleast one antibiotic. The overall resistance rates were generally low for gentamicin, cefixime and ceftazidime respectively. However, penicillin had the highest resistance (100%).

The study focused on the prevalence and antibiogram of bacteria causing URTIs among people in a rural health centre. Respiratory tract infection is considered as one of the most important infectious diseases in developing countries. *Staphylococcus aureus* is an increasingly deadly pathogen that is known to cause a variety of nosocomial and community-acquired infections [18, 19]. In our study, *Staphylococcus aureus* was the commonest pathogen seen in majority of the population (45.61%). *Staphylococcus aureus* has shown a disconcerting propensity to develop resistance to antimicrobial agents and has become an important challenge for the clinicians [20, 21]. Hence, before selecting an antibacterial, the clinician

must first consider the likely causative organism. Knowledge of the prevalent organisms and their current sensitivity is of great help in choosing an antibacterial.

In a study on the epidemiology of respiratory tract bacterial pathogens carried out by Varotto *et al*. (2001), *P. aeruginosa* has been reported as the most prevalent organism (24%) followed by *S. pyogenes* (18%), *S. aureus* (17%) and *K. pneumoniae* (8%) [22]. In an Indian study carried out by Kumari *et al*. (2007) on bacterial isolates from respiratory tract of ICU patients, the percentage isolation rate for *P. aeruginosa*, *Klebsiella* spp., *Enterobacter* spp. have been reported 21.5, 19 and 8 respectively [3].

As shown in Table 1, the bacterial isolates in order of the frequency in our study were *S. aureus* (45.61%), *β hemolytic streptococci* (22.81%) and *Klebsiella pneumoniae* (14.91%), which is not comparable to the two above mentioned studies.

The results of the antibiotic susceptibility study generally revealed that gentamicin and cefixime were the most effective antibiotics against the isolates (Table 1). *S. aureus* showed a sensitivity rate of 97.11% to gentamicin while *β hemolytic streptococci* and *K. pneumoniae* each recorded 96.15% and 97.06% respectively. The major selective force favouring the emergence of antibiotic resistance is their extensive use [23]. It is noteworthy that gentamicin and cefixime is probably less abused than other antibiotics because of its mode of administration (solely by injection) and the prohibitive cost of procurement. These factors may be responsible for the low rate of resistance recorded for these drugs. The high rate of resistance to penicillin (100%) and co-trimoxazole (76.8%), which are commonly bought over-the-counter in drug stores, contrasts with the marked levels of susceptibilities of the isolates to gentamicin, cefixime, and ceftazidime, which are less-frequently used, thus suggesting a relationship between antibiotic use and the level of drug resistance encountered in this study as previously suggested in another study [23].

The increasing frequency of antibiotic resistance has been reported first in infections at sites where penetration of the antimicrobial agent is restricted and the level of therapeutic concentrations is consequently more difficult to be achieved. It could also hinder the eradication of infections in respiratory tract infections treated using standard antibiotic therapy regimens [9]. Accurate information on local epidemiology and antimicrobial resistance patterns of pathogens is

essential to select a clinically effective antibiotic therapy for the infections [24].

Table 1: Prevalence and antimicrobial susceptibility (%) of bacterial strains recovered from throat swabs

Bacterial strains	N (%)	*P	CRO	E	GN	AM	AN	CP	CFM	C	COT	CAZ
S.aureus	104 (45.61%)	0	31.73	28.84	97.11	0	34.62	40.39	99.04	76.92	0	94.2
β haemolytic streptococci	52 (22.81%)	0	44.23	46.15	96.15	48.08	25	38.46	92.31	82.69	38.46	88.46
K. pneumoniae	34 (14.91%)	0	5.88	73.53	97.06	44.12	2.94	73.53	91.18	61.76	29.41	88.24
P.aeruginosa	19 (8.33%)	0	5.26	47.37	84.2	0	52.63	78.95	89.47	63.16	26.32	78.95
α haemolytic streptococci	11 (4.83%)	0	36.37	54.55	90.91	9.09	18.18	45.45	81.82	63.64	27.27	72.73
E.coli	5 (2.19%)	0	20	60	80	0	40	20	100	100	20	80
H.influenzae	3 (1.32%)	0	33.33	66.67	100	33.33	66.67	33.33	100	66.67	0	66.67

n=228

*P = Penicillin; CRO = Ceftriaxone; E = Erythromycin; GN = Gentamicin; AM = Ampicillin; AN = Amikacin; CP = Ciprofloxacin; CFM = Cefixime; C = Chloramphenicol; COT = Co-trimoxazole; CAZ = Ceftazidime

CONCLUSION

Most of the isolates had a high level of resistance to examined antibiotics. There are many possible reasons for this alarming phenomenon, including inappropriate and incorrect administration of antimicrobial agents in empiric therapies and lack of appropriate infection control strategies. This problem indicates importance of performing antibiotic susceptibility testing before empirical therapy.

Surveillance of bacterial infections and monitoring their antimicrobial susceptibility pattern must be carried out not only in tertiary hospitals, but also in small hospital settings. Changing the antibiotic of main prescription periodically (every 6months/1 year) akin to crop rotation to increase soil fertility, could keep resistant organisms at bay in the wards [25].

This study was done only for outpatients. Further study should be carried out for inpatients in surgery, medicine, wounds and post operative cases for best results. These types of data will help in designing and validating the accuracy of guidelines for empirical treatment of upper respiratory tract infections.

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