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ANTIBIOGRAM ASSESSMENT AGAINST UPPER RESPIRATORY TRACT INFECTION CAUSING BACTERIA

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ABSTRACT

Human body is a perfect natural habitat for numerous microorganisms like bacteria, fungi, yeasts and some viruses which are termed as microflora of a body. Amongst its upper respiratory tract, is the most common infections in the world. Our study aimed at determining the antimicrobial resistance of bacterial strains against several antibiotics, isolated from the respiratory tract of the suspected patients. The study was confirmed using in vitro antimicrobial disk diffusion method. Total of 30 samples were studied. 20 bacterial strains were isolated of which 15 were Gram positive bacteria, including *Staphylococcus aureus* (7 strains), *Staphylococcus epidermidis* (4 strains) and *Corynebacterium diphtheria* (4 strains) whereas 5 strains of Gram negative bacteria, includes *Neisseria meningitidis*. The resistance determined by the antibiotics used as, amoxicillin, *Staphylococcus aureus* (87.71%), *Staphylococcus epidermis* (85.71%), *Corynebacterium diphtheria* (75%), erythromycin, *Staphylococcus epidermidis* (100%), *Corynebacterium diphtheria* (100%), *Neisseria meningitidis* (60%), *Staphylococcus aureus* (57.14%); tetracyclin, *Staphylococcus epidermidis* (50%), *Corynebacterium diphtheria* (50%), *Neisseria meningitidis* (40%), *Staphylococcus aureus* (29%); ciprofloxacin, *Neisseria meningitidis* (80%), *Staphylococcus aureus* (29%); penicillin showed 100 % resistance towards all the isolates. The susceptibility of S. aureus was highest among all the isolates. Among the antibiotics Penicillin had the highest resistance. Most of the isolates were multi-drug resistance towards various antibiotics. The ability of the antibiotics to inhibit the growth of bacterial isolates indicated that they are effective antimicrobial agents in upper respiratory tract infections.

INTRODUCTION: The upper respiratory tract (URT) or upper airway primarily refers to the parts of the respiratory system lying outside of the thorax or above the sternal angle.

The upper respiratory tract comprises the conjunctiva, nose, paranasal sinuses, middle ear, nasopharynx, oropharynx and laryngopharynx, which serve as gateways to the trachea, bronchi and pulmonary alveolar spaces. It is largely covered with ciliated columnar epithelium.

Exceptions are the oropharynx, vocal cords, upper posterior epiglottis and mastoid antrum of the middle ear, which are lined with stratified squamous epithelium. The conjunctiva is also composed of stratified squamous epithelium, continuous with and similar to the epithelium of the cornea [1]. Human body is perfect natural habitat for number of microorganisms like bacteria, fungi, yeasts and some viruses which are termed as microflora or resident flora or normal flora of a body.

Upper respiratory tract infections (URTI) are amongst the most common infections in the world. The infection commonly referred as URI or URTI, is an acute infection. Based on the URT the illness includes tonsillitis, pharyngitis, laryngitis, sinusitis, otitis media, and the common cold [2]. This infection is the leading cause of morbidity and mortality in critically ill patients in developing countries.

The respiratory tract is a frequent site of infection because it comes in direct contact with the physical environment and is exposed to airborne microorganisms, including viruses, bacteria, fungi and parasites. Acute respiratory infections accounts for 20-40% of outpatient and 12-35% of inpatient attendance in a general hospital [3]. URTI is the most common bacterial disease affecting people of all ages from the neonate to the geriatric age group.

Upper respiratory tract flora include many gram positive and gram negative bacteria including anaerobic and microaerophilic group of *Streptococci* (found in the sinuses), *Streptococcus pneumoniae*, *Streptococci. S.* '*milleri*' *Haemophilus influenza***,** *Diphtheroids, Streptococcus pyogenes.* Coagulase-negative *Staphylococci*, *Staphylococcus aureus*, *Moraxella catarrhalis, Neisseria* spp, *Prevotella melaninogenicus* and related species [4].

Actinomyces, Bacteriodes, Micrococcus, Enterococcus, Bordetella, Corynebacterium, Fusobacterium, Haemophilus, Lactobacillus, Mycobacterium, Neisseria, Staphylococcus, Streptococcus, Treponema, Klebsiella, Enterobacter, Lactobacillus, Escherichia, Proteus and *Veillonella* also constitute normal flora of throat. Pathogenesis of URTI involves complex interactions between an organism, the environment and the potential host. Management of URTIs is based on information about the host, the organism, and the antimicrobial agent [5]. The effective antibiotics against the different microorganisms for a speedy recovery of the infection are used. A definitive diagnosis is made based on the sensitivity of the particular antibiotics towards the infection. Streptomycin, penicillin, tetracycline, cephalosporin, ciprofloxin, erythromycin, amoxicillin etc are some of the common antibiotics used for the prevention [6].

Pathogens attacking almost all the parts of body showed antibiotic resistance in lesser or greater extent. In the recent year a shift from narrow spectrum to broad spectrum antibiotics by the doctors was reported and increase of bacterial isolates resistant to former antibiotics was blamed for such a shift in practices.

The present study was carried out to identify the bacterial isolate from the upper respiratory tract obtained from the suspected patients and to determine the antimicrobial susceptibility against several antibiotics [7] and also to get rid of multiple drug resistance problems. The aim of this study is to determine drug activity against bacteria of throat infections.

MATERIALS AND METHODS:

Specimen Collection: Random sampling was done from People's Dental College and Hospital and Gandhi Medical College and Hospital, Bhopal. Throat samples were collected with sterile cotton swabs from the actual infection site aseptically into the sterile container containing normal saline. A throat swab will capture the causative microorganism and allow the specific microorganism to be grown in the microbiology laboratory under specific conditions. A sufficient quantity of specimens was obtained to perform the culture technique requested. A total of 50 samples were collected for the study.

Isolation of Microorganism from Clinical Samples: The samples were inoculated on basal and differential media. Plates were observed after overnight incubation for colony characteristics. The isolated colonies of microorganisms were sub-cultured and pure cultures were obtained. Colony characteristics and Gram's technique were used for identification and differentiation. The bacterial cultures were characterized using morphological, microscopy and biochemical tests following the standard procedures.

Identification of Bacterial Isolates: The bacterial cultures were isolated on the basal culture medium and differentiated on the selective medium for further identification. Mannitol salt agar is a common selective media for most of the species of *Staphylococcu*s genus. All the microorganisms were analyzed by biochemical tests such as IMViC, catalase, urease, oxidase, bile

solubility and carbohydrate fermentation test. *Staphylococcus aureus* was identified by the positive catalase, from IMViC-MR positive and acid-gas production by carbohydrate fermentation test, and other biochemical characters. *Staphylococcus epidermis* isolated on and identified by the positive catalase, IMViC (MR) and carbohydrate fermentation tests. *Cornebacterium diphtheria*, isolated on Dextrose Proteose Peptone Agar (DPPA), showed positive results of catalase and carbohydrate fermentation. *Neisseria meningtidis*, isolated on Chocolate Agar, showed positive results of catalase, oxidase and carbohydrate fermentation.

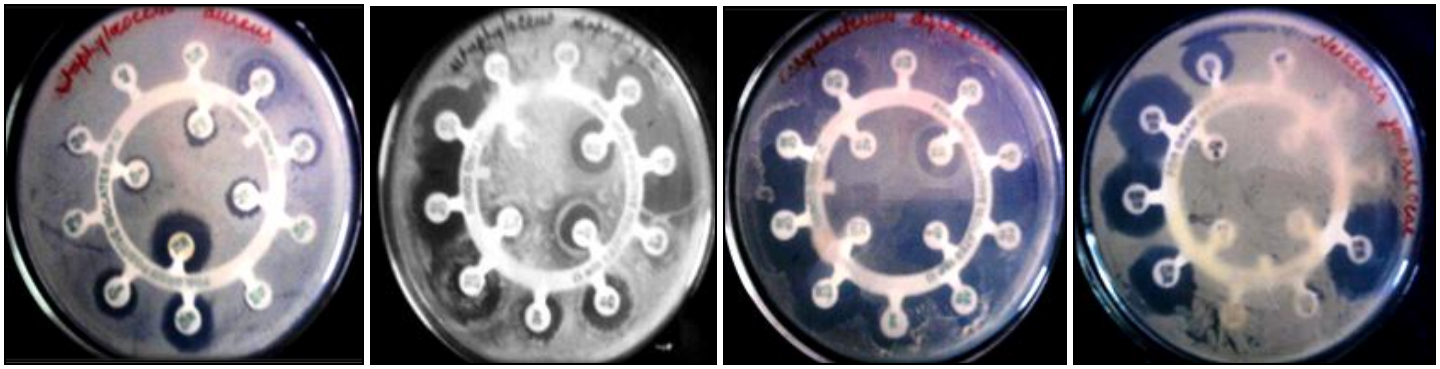
Antibiotic Susceptibility Assay: The antibacterial susceptibility testing of the isolates was done using the Kirby-Bauer disk diffusion method [8]. A small inoculum of each bacterial isolate was inoculated on Mueller-Hinton plates and antibiotic discs of the following drug contents- amoxicillin, erythromycin, tetracycline, ciprofloxacin and penicillin were placed on the plates, spacing them well to prevent the overlapping of inhibition zones. The plates were incubated at 37^{o}C for 24 hours, and the diameters were recorded to determine susceptibility or resistance of the isolated microorganism towards each selected antibiotics [9].

RESULTS AND DISCUSSION: The results depicted in the study revealed the reactivity of the antibiotics on the microorganisms. **Table 1** represents the resistance of selected antibiotics towards the experimental microorganisms.

Fig. 1 shows the resistance of experimental microorganisms towards the selected antibiotics by disk diffusion or Kirby-Bauer method. The susceptibility towards amoxicillin was least of *S. aureus* (12.29%), *S. epidermis* (14.29%) and *C. diphtheria* (35%). In series was erythromycin *N. meningitides* (40%), *S. aureus* (42.86%), whereas *S. epidermis* and *C. diphtheria* showed no results of susceptibility. *S. epidermis* (50%) and *C. diphtheria* (50%) have same susceptibility towards tetracycline than was *N. meningitides* (60%), *S. aureus* (71%). Ciprofloxacin showed least towards *N. meningitides* (20%), *S. aureus* (71%) and no susceptibility for *S. epidermis* and *C. diphtheria*. Among all the antibiotics susceptibility of all the isolates towards penicillin was nil.

TABLE 1: ANTIMICROBIAL RESISTANCE PATTERN OF UPPER RESPIRATORY TRACT INFECTION

Bacteria	Strains	Antibiotics Resistance Strains (%)				
		Amoxicillin	Erythromycin	Tetracycline	Ciprofloxacin	Penicillin
S. aureus	7	87.71%	57.14%	29%	29%	100%
S. epidermis	4	85.71%	100%	50%	-	100%
C. diphtheria	4	75%	100%	50%	-	100%
N. meningitides	5	-	60%	40%	80%	100%



A **B** **C** **D**

A: Antimicrobial activity against *S. aureus;* B: Antimicrobial activity against *S. epidermis; C:* Antimicrobial activity against *C. diphtheria;* D: Antimicrobial activity against *N. meningitidis*

FIG. 1: *IN VITRO* ANTIMICROBIAL ACTIVITY BY DISC DIFFUSION METHOD

Upper respiratory tract infections (URTIs) are the most common human infections. Management of patients with otitis media, sinusitis or pharyngitis is based on information about the host, the organism and the antimicrobial agents [10]. A large number of bacterial species colonize the upper respiratory tract (nasopharynx). The nares (nostrils) are always heavily

colonized, predominantly with *S. epidermis* and *Corynebacteria,* and often (in about 20% of the general population) with *S. aureus,* this being the main carrier site of this important pathogen. Sometimes pathogens such as *Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae* and *N. meningitidis* colonize the pharynx. In older times hemolytic streptococcus was considered the commonest causative organism of throat infection but now the trend is changing and other aerobic and anaerobic organism are coming in lime light. Every microorganism has its own sensitivity and resistance profile towards a particular antibiotic. The resistance and susceptibility of all the microorganism depends on its metabolic activities and enzymes. Staphylococcal resistance towards the different antibodies is mediated by an extracellular enzyme β-lactamase. The enzyme is activated when the microorganism is exposed to β-lactam antibiotics. The enzyme hydrolyses the β-lactam ring and shows highest resistance towards the antibiotic [11]. The study revealed that *S. aureus* was most susceptible towards tetracycline and ciprofloxacin than towards erythromycin and amoxicillin. *S. epidermidis* and *C. diphtheria* most for tetracycline and than to amoxicillin and were non susceptible to erythromycin. *N. meningitidis* were most susceptible to tetracycline than to erythromycin and ciprofloxacin. Among all the antibiotics penicillin was most resistance towards all the isolates. The selective antibiotics and their extensive use increase their resistance. A relationship between antibiotic and a marked level of resistance towards all the isolates is encountered in this study.

CONCLUSION: Upper respiratory infections are the major causes of morbidity and mortality worldwide, being much more common in temperate and cold than in hot climate. It occurs both among children and adults [12]. In the present study, we have found Staphylococcus spp., *C. diphtheria* and *N. meningitides* was observed in throat sample. Drug targets/antibiotics should be developed-synthesized which can overcome the throat infection caused by these isolates. The study was conducted with aims to identify the causative bacterial agents of upper respiratory tract infection (URTI) among pediatric patients and to reveal the antibiotic susceptibility pattern of the major pathogen. These data demonstrate that the continued evolution and geographical variation in bacterial resistance, highlights the need of appropriate antimicrobial agents. The frequency of the drug resistant bacteria has increased as compared to the previous years and it is an important issue to be addressed.

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