International Journal of Advanced Research in Biological Sciences

www.ijarbs.com

Research Article

Microbiological analysis and Antibiogram of different human Respiratory infections

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Abstract

In order the study normal flora, the samples were collected from non infected individuals. In order the study the causative agents, the sample were collected form infected individuals. Sample was processed both macroscopically and microscopically. The results revealed that the normal flora of throat of individuals without throat infections (14 specimens) were *Staphylococus aureus*. Among 110 specimens collected from individuals with throat infections, 47 were positive for *Staphylococcus aureus* and 12 were positive were positive for *Staphylococcus epidermidis*. And among the remaining specimens, 39 were *Pseumonas aeruginosa*, 6 were Proteus mirabilis, 4 were *Klebsiella pneumoniae* and 2 were gram positive cocci in chains (*Streptococci).* All bacteria were tested for their susceptibility to different antibiotics. Among 47 *Staphylococcus aureus*, 33 were sensitive to penicillin and 14 were resistant. Fungus such as *Candida albicans*, *Candida tropicalis* and *Aspergillus niger* were isolated and identified. This kind of infection reflects the immunodeficiency of the individuals.

Keywords: *Staphylococus aureus* ; throat infections ; penicillin; *Candida albicans.*

Introduction

There are a number of acute and chronic infections that can affect the lower respiratory tract, the focus of this paper is to look at the two most common infection, Bronchitis and pneumonia, as identified by Therapeutic guidelines. Although influenza affects the lower respiratory tract, it also affects the upper respiratory tract and hence it will not be discussed here. Antibiotics are often thought to the first line treatment in lower respiratory tract infections however as discussed later these are not indicated in viral infections. It is important to use appropriate antibiotic selection based on the infecting organism and to ensure this therapy changes with the evolving nature of these infections and the emerging resistance to conventional therapies. *Haemophilus influenzae* and *M catarrhalis* are of increasing importance in both community acquired pneumonia (CAP) and acute exacerbation of chronic bronchitis (AECB) while the importance of *S.pneumoniae* is declining. It has also become apparent the importance of atypical pathogens such as *k.pneumoniae, M.pneumoniae* and *L.pneumonia*, in CAP.

Fungal respiratory disease consists of fungal colonization, allergy and infections of respiratory tract and lungs. Infectious fungal respiratory diseases remain important causes of morbidity and mortali9ty in immunocompromised individuals, especially those marrow transplantation or solid – organ transplant (SOT) and those with HIV infection. Previous review articles have emphasized the advance in invasive pulmonary aspergillosis (IPA) allergic bronchopulmonary spergillosis. In this article, we try to describe the recent advance in aetiology, including the common and uncommon causative organisms of infectious fungal respiratory diseases, summarize briefly the clinical presentations, list and evaluate the diagnostic tools used in the past year, and review the antifungal treatment and prophylaxis of the important fungal respiratory infections, such as IPA and mucormycosis.

Lower respiratory tract infections (LRTI) are a common reason for consulting general practitioners (GPs). In most cases the aetiology is unknown, yet most result in an antibiotic prescription. The aetiology of LRTI was investigated in a prospective controlled study. The most common organisms identified in the patients were rhinoviruses (33%), influenza viruses (24%), and *Streptococcus pneumoniae* (19%) compared with 2% ($p<0.001$), 6% ($p =0.013$), and 4% ($p =0.034$), respectively, in controls. Multiple pathogens were identified in 18 of the 80 LRTI patients (22.5%) and in two of the 49 controls (4%; $p =0.011$). Atypical organisms were rarely identified. Cases with bacterial aetiology were clinically indistinguishable from those with viral aetiology. Patients presenting to GPs with acute adult LRTI predominantly have a viral illness which is most commonly caused by rhinoviruses and influenza viruses (Creeer *et al.*, 2006).

We determined the frequency of *Streptococcus pneumoni*ae, *Haemophilus influenzae, Staphylococcus aureus,* and Gram-negative enteric bacteria (GNEB) in pneumonia patients, determined the antibiograms of these pathogens, and investigated the relationship between pneumonia and selected risk factors. (GNEB) infection was prevalent (47%) over 70 years old. Gentamicin and levofloxacin were the most effective against these bacteria (Nagalingam *et al.,* 2005)

Smoking is a risk factor for a number of pulmonary infections, probably because of its adverse effects on respiratory defences. It is associated with increased morbidity and mortality from pneumonia and influenza, as well as more days lost from work from lower respiratory infections. Patients who smoke need to be informed about their increased risk of respiratory infections and of the benefits of both being vaccinated and stopping smoking (Murin and Bilello, 2005).

In order to determine the current antibiotic susceptibility of *Streptococcus Pneumoniae* strains in Greece, the present study was performed on 282 clinical isolates collected from patients at the sotiria chest Hospital of Athens, Greece, during the years 1997 -2003. Susceptibility testing revealed that 52 (18.4%) isolates were not susceptible to penicillin, with 13.1% demonstrating intermediate and 5.3 % high – level resistance. One of the penicillin non-susceptible isolates was also resistant to cefotaxime. Comparison with results of a previous study conducted at the same hospital during the period 1992 – 1993 showed that penicillin resistance had increased by 4.5 %. The results of this study indicate the antimicrobial susceptibility of *Strptococcus pneumoniae* continues to change in Greece and continuous surveillance remains important for guiding empirical antibiotic therapy (Kanavaki *et al*..2005).

The study done with the following aims and objectives To analyze the throat samples collected from different patients admitted in Vandavasi Government Hospital with respiratory ailments. To identify the bacterial and fungal pathogen and to study their antibiogram If the isolates are identified to the susceptible to be susceptible to those antibiotics, then it would be possible to pass it to the medical community to use them to overcome the problem of the pathogenic isolates in a better

manner compared to the present status, after fulfilling the safety standards.

Materials and Methods

Sample collection

In this study throat swab samples were collected from different patients admitted in Vandavasi Government hospital. The bacteria and fungi were isolated and identified from the patients and analyzsed for their susceptibility to common antibiotics and two selected herbal extracts.

For collection of materials sterile cotton swab were used. Sample was obtained with the swab with the minimum of contamination and it was immediately put into the sterile test tube containing few drops of saline in order to prevent drying of the swab. Two samples were collected from each patient. Then each specimen was labelled with the particulars such as name and age of the patient and if any chemotherapy he/she had previously and the social status of his /her patients.

Microscopic Examination

Staining

The organisms grown on culture plates were stained using gram-staining method. A thin smear was .prepared on the medium it was flooded with crystal violet solution and allowed to stand for one minute. Then it was washed with water and then flooded with gram's iodine solution. It was drained and decolorized with 95 % ethanol, which then washed gently in running water. Then the same was stained with a counter stain called safranine for 30 seconds. After drying the stained smear was observed under microscope to identify the organisms and to place them in their own particular group by their individual reaction to certain stains as gram positive or gram negative rods or cocci.

Motility test

The hanging drop technique was followed to observe the motility of the bacteria with which they are classified as motile and non –motile organisms. A drop of suspension of culture was placed at the center of a cover slip and placed in an inverted position over a cavity slide so that the drop is hanging over the cavity. The edge of the drop was observed under low power lens of a microscope where the darting or corkscrew movement of the organisms can be seen. The results were noted.

Isolation of bacteria from samples

The remaining one swab was touched on the prepared nutrient agar, blood agar and Mac Conkey agar plates aseptically. Then by using inoculation needle, it was streaked for the growth of isolated colonies and then the plates were incubated at 37ºc for 24 hrs for bacteria. After 24 hrs the colonies and grown on the plates were examined for their morphology, haemolytic and the same type of colonies was used for grams staining. The pink color colonies in MacConkey medium indicate lactose fermentation i. e., positive reaction. The results were tabulated.

Morphology

The morphology of each type of colony was examined and the results were noted.

Staining

Each type of colony was aseptically taken from the plate and a thin smear was prepared on a glass slide. The preparation was heat fixed, gram stained and observed under microscope. The results were noted.

Subculture

The same type mof colony was simultaneously taken from the plate aseptically and streaked on the prepared nutrient agar plate. Then the plates were incubated at 37 C for 24hours. After one day, the results were noted for their colony morphology and pigment production and also the colonies grown on the plate were used for performing biochemical tests and antibiotic sensitivity test.

Test for *Staphylococci*

0.5 ml of diluted (1:4) citrated human plasma in a small sterile tube was inoculated with heavy saline suspension of the organism and was incubated at 37º C for 1 -4 hours. It was examined every 15 minutes for the formation of coagulum compared with the controls. The results were noted.

Selective media

The suspected colony was taken aseptically and streaked on Baird parker agar and mannitol salt agar medium and were incubated at 31º C for 24 hours. After one day the results were examined for their colony morphology and colour. The results were tabulated.

Biochemical tests for identification

Sugar fermentation test

The term sugar in microbiology denotes any fermentable substance. They may be monosaccharides, diasaccharides, polysaccharides, trisaccharides, alcohols, glycosides and noncarbohydrate substances such as inositol.

The sugars were fermented by various type of bacteria and in that process acid and gas was produced which was indicated by the colour indicated by the indicators for acid and gas collected in to the inverted Durham‘s tube was kept inverted in the sugar tube to detect gas production. For organisms exacting in their growth requirements (e.g. *pneumococci)* Hiss’ serum sugars were used. The incubation period was 24 hours and the temperature was generally 37 º C.

The organisms grown in the plates were inoculated in the prepared different sugar media containing glucose, sucrose lactose and mannitol and were incubated for 24 hours at 37 º C.

Indole test

The colony from the plate was inoculated into the indole nitrate medium in a tube and incubated at 37 ºC for 24 hours. The formation of red ring upon the addition of Kovac’s reagent indicates the positive reaction.

Methyl red test

The colony from the plate was inoculated into MR – VP broth tubes and incubated at 37 ºC for 24 hours. The formations of red colour on the addition of methyl red indicator indicate the positive reaction.

Voges –proskauer test

The same type colony was inoculated at 37ºC for 24 hours. Development of pink to bright red colour on the addition of Barrit’s reagent A and B to the medium indicates positive reaction.

Citrate utilization test

Slant of Simmon’s citrate agar medium was inoculated with the organism grown on the plate and incubated 37ºC for 24 hours. Change of the colour from green to blue indicates the positive reaction.

Catalase production

Add a loopful of 10 % H_2O_2 on colonies on nutrient agar. Prompt brisk effervescence indicates catalase production.

Oxidase test

The organisms taken from the plate was streaked on filter paper incorporated with oxidase reagent (1% tetramethyl paraphenylene diamine dihydrochloride in water) the appearance of purple colour indicated positive result.

Urease test

This test is done in urease medium. Inoculated the slope heavily and incubated at 37 ºC. Urease positive culture produces a purple pink colour.

Gelatin hydrolysis

The colony to be tested was stabbed into gelatine deep tubes. The tubes were incubated at 37ºC for 24 hours. Then the tubes were kept in the refrigerator

for an half an hour at 4ºC. Cultures that remain liquefied produce gelatinase and shows positive reaction.

Oxidation fermentation test

O –F base was prepared and sterilized at 121 º C for 15 lbs. one gram of carbohydrate in 10 ml of distilled water was sterilized by filtration. After sterilization O –F medium base was cooled to 55 º C and 10ml of sugar solution was added to the medium. Then the medium was distributed in 5ml quantities in 12 100m tubes and allowed to set in a upright position to get the solid butt. Then the test organism was stabbed 3 times and petroleum jelly was poured over the butt and then incubated at 37ºC for 4days. Controls were prepared with and without inoculum.

Fungus identification studies

Microscopy – KOH mount:

A drop of 10% KOH is mixed with the vaginal discharge on a glass slide and coverslip is placed over it and examined for the budding yeast cells directly under objective of the microscope.

Gram's staining:

The throat swab smear was stained by Gram's staining method and observed for Gram positive budding yeast cells.

Culture for *candida*

The samples were inoculated onto Sabouraud's Dextrose Agar with antibiotic (Chloramphenicol) and were incubated at 37 ºC for 24 to 48 hrs for the isolation of Candida. Yeast colonies in the culture were identified by colony morphology Gram 's stain, germ tube formation , chlamydospore formation, sugar fermentation and assimilation test (Bailey and Scott)

Speciation of *candida*

The conventional method for speciation of *Candida*, carbohydrate fermentation and assimilation test was developed by Wickerham and Barton.

Chlamydospore formation test

Pure isolated colony of yeast was inoculated in corn meal agar and incubated at 25 ºC for 48 hrs and then examined microscopically for the formation of thick double wall, spherical chlamydospore.

Carbohydrate assimilation test

To 1.5 ml of yeast nitrogen base sugar free media was added, allowed to solidify. Disc impregnated with 20 % sugar solution was placed on the agar incubated at 25 º C for 24 – 72 hrs and examined for the zone of growth around each disc. Species were identified according to their difference in assimilating the sugars.

Carbohydrate fermentation test

To the fermentation broth with 1 % respective 50 micro litre of the saline suspension of inoculum grown on sugar free media was inoculated and incubated at 37ºC for 24 hrs to 48 hrs and checked for acid / gas production. Species were identified by their patterns of fermentation of sugars.

Method

The isolates were sub cultured on to Sabouraud Dextrose Agar (SDA) and incubated at 37ºC overnight. Saline suspension of the organisms was made and the turbidity was adjusted to 0.5 Mc Farland standards. A lawn culture was done on to the freshly prepared yeast nitrogen base plates. The discs were placed and the plates were incubated at 37º C. The zone of inhibition was recorded after 24 hrs and 48 hrs. The isolates were classified as susceptible, intermediate and resistant based on diameter of the zone of inhibition.

Germ – tube test medium

Serum is suspended (0.3 to 0.5 ml / tube) aseptically into tubes (12 X 7 mm) with plastic caps and

overnight culture was inoculated and incubated for 3 hrs at 37º C. For every 30 mins the tube is taken out and shaken mildly and reincubated in the same environment. After 3 hrs the content of the tube is analyzed for yeasts with germ tubes.

Sterilized by filtration and dispensed aseptically

10ml of the above solution was added to 90 ml 2 % molten agar and plates were poured.

Results and Discussion

Characterization of *Staphylococcus aureus*

Microscopy : G + ve cocci with more pus cells
Colony morphology : Circular , convex , smooth , Golden yellow colour colony in Nutrient agar, Beta haemolytic colony in Blood agar, yellow coloured colonies in Mannitol salt agar was observed (Table.1).

Characterization of *Streptococcus pyogens*

Microscopy : Gram positive cocci in chains
Colony morphology : White colour colonies in nutrient agar .Alpha – haemolytic in blood agar, Beta fermenting in MacConkey agar was observed (Table.2).

Characterization of *Pseudomonas aeruginosa* (Table. 3)

Microscopy : G – ve Rod with more pus cells
Motility Test and
Endospore Staining : Motile & Negative
Cultural characters : Large, Opaque, irregular colonies with a distinctive, Musty or earthy smell.
On Nutrient Agar : Green colour colony with diffused pigmentation
On Mac Conkey agar : Forming NLF colonies
On Blood agar : Beta haemolytic colonies
Triple Sugar Iron Test : Alkaline butt – Alkaline slant

Characterization of *Klebsiella Pneumoniae* (Table.4)

Microscopy : G – ve Rod (diplobacilli) with a few pus cells
Motility Test : Non- Motile
Capsule staining : Positive
Endospore Staining : Negative
Colony morphology : Large Mucoid colonies
On MacConkey : Pink colour (LF colonies) Mucoid colonies
On Blood agar : alpha haemolytic colonies
Triple Sugar iron agar Test : Acid butt – Acid slant,
Gas+ ve
H_2S +ve

The results revealed that the normal flora in the throat of individuals without respiratory infections were *Streptococcus epidermidis*. It indicates that the major skin commensal (*Staphylococcus epidermidis)* is also the oral commensal.

Among 110 specimens from patients with respiratory infections, 47 were positive for *Staphylococcus aureus* and 12 were positive for *Staphylococcus epidermidis*. This reveals that *Staphylococcus epidermidis* was an opportunistic pathogen which was, though present as normal flora, also causing this disease. And among the remaining specimens, 39 were *Pseudomonas aeruginosa*, 6 were *Proteus mirabilis*, 4 were *Klebsiella pneumoniae* and 2 were positive for gram positive cocci (*Streptococci*) in chains.

All bacteria were tested for their susceptibility to different antibiotics. Among 47 *Staphylococcus aureus*, 33 were sensitive to penicillin and 14 were resistant. About 7 *Staphylococcus aureus* were found to be resistant to methicillin. Such resistant organisms were susceptible to chloramphenicol and septra (Co – trimaxazole) Gentamycin. All *Pseudomonas aeruginosa* were sensitive to ciprofloxacin. *Klebsiella pneumoniae* was sensitive to chloramphenicol (Tables 7 -10).

Table.1 Biochemical characterization of *Staphylococcus aureus*

S. No.	Name of the test	Result
1	Catalase	Positive
2	Oxidase	Negative
3	Coagulase	Positive
4	Indole	Negative
5	Methyl red	Positive
6	Voges Proskauer	Positive
7	Citrate	Positive
8	Urease	Positive
9	Gelatinase	Positive
10	Nitrate	Positive
11	Mannitol fermentation	Positive

Table. 2 Biochemical characterization of *Streptococcus pyogens*

S . No.	Name of the Test	Result
1.	Catalase	Negative
2.	Oxidase	Negative
3.	Coagulase	Negative
4.	Indole	Negative
5.	Methyl red	Negative
6	Citrate	Negative
7	Urease	Negative
8	Gelatinase	Negative
9	Nitrate	Negative
10	Mannitol fermentation	Positive

Table. 3 Biochemical characterization of *Pseumonas aeruginosa*

S . No	Name of the Test	Results
1.	Catalase	Positive
2.	Oxidase	Positive
3.	Coagulase	Not Done
4.	Indole	Negative
5.	Methyl red	Positive
6.	Voges proskauer	Negative
7.	Citrate	Positive
8.	Urease	Positive
9.	Gelatinase	Positive
10.	Nitrate	Positive

Table.4 biochemical characterization of *Klebsiella pneumoniae*

S. No.	Name of the Test	Result
1.	Catalase	Positive
2.	Oxidase	Negative
3.	Coagulase	Not Done
4.	Indole	Negative
5.	Methyl red	Negative
6.	Voges proskauer	Negative
7	Citrate	Positive
8.	Urease	Positive
9.	Geltinase	Positive
10.	Nitrate	Positive
11.	O \F Test Glucose	Fermentative

Table.5 Carbohydrate fermentation test for the *Candida* isolates

S. No	Species	Sugar Fermentation			
		Dextrose	Maltose	Sucrose	Lactose
1.	*Candida albicans*	AG	AG	A/ -Ve	-Ve
2.	*Candida tropicalis*	AG	A G	AG	-

Table.6 Carbohydrate assimilation test for the *Candida* isolates

S. No	Species	Sugar fermentation						
		Dext	Mal	Sucr	Lact	Gala	Rafi	Cellu
1.	*Candida albicans*	+	+	+	-	+	-	-
2.	*Candida*	+	+	+	-	+	-	+

Table.7 Antibiotic sensitivity for *Staphylococcus aureus*

S. No	Name of the antibiotic	Diameter of Zone (Result in mm)
1.	Ampicillin	32 mm(S)
2.	Amoxycillin	21 mm (S)
3.	Cefotaxime	07 mm(R)
4.	Ceftriaxone	15 mm(S)
5.	Cephalexin	19 mm(S)
6.	Ciprofloxacin	26 mm(S)
7.	Cloxacillin	18 mm(S)
8.	Co - trimaxazole	21 mm(S)
9.	Erythromycin	08 mm(S)
10.	Gentamycin	14 mm(I)
11.	Penicillin	05 mm(R)
12.	Norfloxacin	22 mm(S)
13.	Ofloxcin	10 mm(R)
14.	Methicillin	12 mm(R)
15.	Tetracycline	22 mm(S)

(S) – Sensitive (I) – Intermediate R) – Resistant

Table. 8 Antibiotic sensitivity for *Streptococcus Pyogenes*

S. No	Name of the Antibiotic	Diameter of Zone(Result)
1.	Ampicillin	32 mm(S)
2.	Amoxycillin	21mm(S)
3.	Cefotaxaxime	13 mm(S)
4.	Ceftriaxone	15 mm(S)
5.	Cephalexin	19 mm(S)
6.	Ciprofloxacin	26 mm(S)
7.	Cloxacillin	18 mm(S)
8.	Co – trimaxazole	21 mm(S)
9.	Erythromycin	16 mm(S)
10.	Gentamycin	14 mm(S)
11.	Penicillin	15 mm(S)
12.	Norfloxacin	22 mm(S)
13.	Ofloxcin	17 mm(S)
14.	Methicillin	18 mm(S)
15.	Tetrtacycline	22 m(S)

(S) –Sensitive (I) –Intermediate (R) – Resistant

Table.9 Antibiotic sensitivity for *Pseumonase aeruginosa*

S. No	Name of the Antibiotic	Diameter of Zone (Result in mm)
1.	Ampicillin	29 mm(S)
2.	Amoxycillin	24 mm(S)
3.	Cefotaxime	15 mm(I)
4.	Ceftriaxone	11 mm(R)
5.	Cephalexin	12 mm(R)
6.	Ciprofloxacin	25 mm(S)
7.	Cloxacillin	Not Used
8.	Co trimaxazole	14 mm(I)
9.	Erythromycin	No Zone(R)
10.	Gentamycin	16 mm(R)
11.	Penicillin	Not Used
12.	Norfloxacin	20 mm(S)
13.	Ofloxcin	No Zone
14.	Methicillin	Not Used
15.	Tetracycline	No Zone

(S) – Sensitive (I) – Intermediate (R) Resistant

Table. 10 Antibiotic sensitivity for *Klebsiella pneumoniae*

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S. No	Name of the Antibiotic	Diameter Zone (Result in mm)
1.	Ampicillin	14 mm(S)
2.	Amoxycillin	12 mm(R)
3.	Cefotaxime	14 mm(R)
4.	Ceftriaxone	16 mm(S)
5.	Cephalexin	17 mm(S)
6.	Ciprofloxacin	24 mm(S)
7.	Cloxacillin	Not Used
8.	Co – trimaxazole	14mm(I)
9.	Erythromycin	No Zone(R)
10.	Gentamycin	15 mm(I)
11.	Penicillin	Not Zone
12.	Norfloxacin	19 mm(S)
13.	Ofloxacin	No Zone(R)
14.	Methicillin	Not Used
15.	Tetracycline	No Zone(R)

(S) –Sensitive (I) – Intermediate (R) -Resistant

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