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Respiratory Bacterial Flora from Healthy as well as Respiratory Symptoms' Subjects

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Abstract: Respiratory system is the primary settlement place of opportunistic organisms and considered as chief carrier of common respiratory pathogens. The aim of the study was to know the opportunistic organisms present in the healthy subjects as well as subjects that were suffering from respiratory symptoms. The organisms were identified as per standard bacteriological protocol and pathogenicity tests of the identified organisms were performed in mouse model. Antibiotic sensitivity of the identified organisms was performed. The bacterial flora present in the throat swab of apparently healthy as well as subjects suffering from respiratory symptoms were: *Staphylococcus* spp. (39.44%) of which Coagulase positive *Staphylococcus* (21.13%) and Coagulase negative *Staphylococcus* (18.31%), *Klebsiella* spp. (19.72%), *Pseudomonas* spp. (15.49%), *Proteus* spp. (4.23%), *E. coli* (9.86%) and *Bacillus* spp. (11.27%). Among the isolates *Staphylococcus*, *Klebsiella* and *Pseudomonas* were the predominant species. Percentages of identified bacteria were higher in respiratory symptoms exhibiting individuals (53.52%) than apparently healthy individuals (46.48%). All coagulase positive *Staphylococcus*, *Klebsiella* spp. and *Pseudomonas* spp. isolated from respiratory symptoms' subjects were found to be pathogenic. The isolated bacteria were resistant to amoxicillin and ampicillin but sensitive to ciprofloxacin and norfloxacin. Isolated *Pseudomonas* spp. showed multidrugs resistant properties. The study provided information about the pathogenic organisms' present respiratory systems of apparently healthy as well as subjects suffering from respiratory symptoms. The pathogenic natures of the isolated organisms were determined to make aware of scientists as well as clinicians. Antibiotics sensitivity assays would provide information to the clinicians for the selection of appropriate antibiotics to treat their patients.

Key words: Opportunistic organisms, respiratory tract, apparently healthy, pathogenicity, antibiotic sensitivity

INTRODUCTION

Respiratory system is the important avenue of bacterial infection. All potential respiratory pathogens primarily adhere to the respiratory mucosa and become infective under favorable circumstances. In this regard, viruses play a vital role to perpetuate such infections (Adderson, 2006). Therefore, respiratory tract is considered as primary settlement site for opportunistic organisms as well as chief carrier of common respiratory pathogens (Belliveau, 1973). It is well known that in immunocompromised hosts, microorganisms present in oral cavity and cause opportunistic infections and systemic diseases such as bacterial endocarditis, aspiration pneumonia and osteomyelitis (Philip *et al*., 2009). Among the bacteria, *Escherichia coli*, *Klebsilla pneumoniae*, *Haemophilus influenza*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris* are the common bacteria of nosocomial infections for respiratory tract infections (Sittiwet *et al*., 2009).

In this regard, Das *et al*. (2010) pointed out that the presence of respiratory pathogen such as Klebsiella might be attributed to the bacterial aerosols generated due to sneezing and coughing in public places. It proves that healthy carrier may transmit the infection to susceptible individuals. It is known that more than 200 species of bacteria colonize on upper respiratory tract (Nadel *et al*., 1999) of which *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Neisseria meningitides*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Haemophilus influenzae* were potential pathogens (Todar, 2011). Recent study suggests that bacteria found in the throat, as well as in the mouth, can be drawn into the lower respiratory tract. This can cause infections or worsen existing lung conditions. People with

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respiratory diseases, such as chronic obstructive pulmonary disease, typically suffer from reduced protective systems, making it difficult to eliminate bacteria from the lungs (AAP, 2011) Treatment of the illness may not be judicious if proper identification of the causal agents is not performed perfectly. Moreover, multidrugs resistant strains are being developed due to indiscriminate use of antibiotics irrespective of the identification of causal agents. Therefore, the present research was undertaken to isolate and identify the bacteria from respiratory tract of apparently healthy and subjects suffering from respiratory symptoms and to study their pathogenicity and antibiotic sensitivity. This would provide us information of the types of bacteria present in respiratory systems during healthy life as well as at stress condition with respiratory symptoms. Moreover, pathogenicity of isolated organisms as well as their antibiotic sensitivity pattern would provide treatment guidelines to the clinicians.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh during the period of January 2009 to December 2009.

Samples: A total of 60 throat swab samples were aseptically collected in which 30 from apparently healthy adult subjects and 30 from respiratory symptoms' adult subjects with the help of sterile cotton buds. Immediately after collection, the samples were inoculated into nutrient broth. These were then transferred to the laboratory, Department of Microbiology and Hygiene BAU, Mymensingh-2202, Bangladesh.

Isolation and characterization of bacteria: The collected samples were processed as per the procedure of Cheesbrough (2006). Briefly the samples were then inoculated into Blood Agar (BA) media and incubated at 37°C for 24 h. Characteristic colonies from the plates were isolated and then sub cultured to obtain pure culture.

The isolated organisms were identified based on colonial morphology, microscopic study and biochemical tests according to standard laboratory methods of Cheesbrough (2006).

Pathogenicity tests: The isolated *Staphylococcus, Klebsiella* and *Pseudomonas* organisms were subjected to pathogenicity test in 2 months old mice by intra-peritoneal inoculation to observe their pathogenicity.

Antibiotic sensitivity tests: Antibiotic sensitivity tests were performed using disc diffusion test of the method described by CLSI (2006).

RESULTS AND DISCUSSION

Bacterial from healthy subjects as well as subjects suffering from respiratory symptoms: The bacteria isolated and identified from the throat swabs of apparently healthy and subjects suffering from respiratory symptoms have been presented in Table 1. It reveals from the Table 1 that 15 were coagulase positive *Staphylococcus* sp. (21.13%), 13 were Coagulase negative *Staphylococcus* sp. (18.31%), 14 were *Klebsiella* spp. (19.72%), 11 were *Pseudomonas* spp. (15.49%), 3 were *Proteus* spp. (4.23%), 7 were *Escherichia coli* (9.86%) and 8 were *Bacillus* spp. (11.27%). Among the 71 isolates 33 (46.48%) were identified from apparently healthy subjects and 38 isolates (53.52%) from respiratory symptoms exhibiting subjects. Similar studies were conducted by Kabra *et al.* (2004), Dedeic *et al.* (2007) and Berkovitch *et al.* (2002). Kabra *et al.* (2004) isolated *Staphylococcus aureus* and *Pseudomonas aeruginosa* and Dedeic *et al.* (2007) isolated *Klebsiella pneumoniae*, *Pseudomonas* spp., *Acinetobacter baumannii* and *Serratia marcescens*. Berkovitch *et al.* (2002) also isolated *Staphylococcus aureus* from throat swab of man. Study conducted by Todar (2011) was showed the predominant normal bacterial flora of respiratory tract of human included *Staphylococcus epidermidis*, *Staphylococcus aureus, Streptococcus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp. and *E. coli*.

Pathogenicity tests: Some of the inoculated mice died within 48 h of inoculation, revealed symptom of labor pneumonia including coughing, sneezing and respiratory distress before death. The other mice did not show any sings of illness and finally survived. No symptoms or death was observed in any of the control group of animal. The animals those died revealed the hemorrhages and congestion of lung after post mortem examination. The organisms were re-isolated from the lung specimens. The results of pathogenicity tests have been presented in Table 2. Torres and Stanislawa (1970) determined the pathogenicity of *Staphylococcus aureus* in mice by injecting through the intra-cerebral and intra-peritoneal routes, Domenico *et al.* (1982) determined the virulence of *K. pneumoniae* by injecting intra-peritoneally into mice and similarly Daniel *et al.* (1992) performed pathogenicity test of *Pseudomonas* spp. through intranasal and intra-peritoneal route. Findings of almost all authors described above are in agreements of the present study.

Table 1: Bacteria isolated from throat swab of apparently healthy and respiratory symptomatic showing subjects

	Health status					
	Apparently healthy		Respiratory symptom's subjects		Total	
Name of isolates	Frequency of isolate	%	Frequency of isolate	%	No.	%
Coagulase positive *Staphylococcus* sp.	3	4.23	12	16.90	15	21.13
Coagulase negative *Staphylococcus* sp.	10	14.08	3	4.23	13	18.31
Klebsiella spp.	5	7.04	9	12.68	14	19.72
Pseudomonas spp.	4	5.63	7	9.86	11	15.49
Proteus spp.	3	4.23	0	0.00	3	4.23
Escherichia coli	4	5.63	3	4.23	7	9.86
Bacillus spp.	4	5.63	4	5.63	8	11.27
Total	33	46.48	38	53.52	71	100.00

Table 2: Results of pathogenicity tests performed in mouse

Name of organisms	Samples from	Number of experimental mice	Route of inoculation	Amount of inoculum (Dosages)	Number of death within 48 h	Pathogenicity
Coagulase positive	AH	3	I/P	0.5 mL	0	NP
Staphylococcus sp.	S	3	I/P	(3.7×10^6 cfu)	3	P
Coagulase negative	AH	3	I/P		0	NP
Staphylococcu	S	3	I/P		0	NP
Klebsiella spp.	AH	3	I/P	0.5 mL (5×10^5 cfu)	0	NP
	S	3	I/P		3	P
Pseudomonas spp.	AH	3	I/P	0.5 mL (2×10^7 cfu)	0	NP
	S	3	I/P		3	P
Nutrient broth (Control)		3	I/P	0.5 mL	0	NP

AH: Apparently healthy man, S: Sick man, I/P: Intraperitoneal, CFU: Colony forming units, P: Pathogenic, NP: Non pathogenic

Table 3: Results of antibiotic sensitivity tests

		Name of isolates					
		Coagulase positive *Staphylococcus*		*Klebsiella* spp.		*Pseudoonas* spp.	
Name of antibiotics	Sensitivity and resistant patterns	No.	%	No.	%	No.	%
PEF	S	2	33.33	6	100	0	00
	I	4	66.67	0	00	0	00
	R	0	00	0	00	6	100
AML	S	0	00	0	00	0	00
	I	0	00	0	00	0	00
	R	6	100	6	100	6	100
AMP	S	0	00	0	00	0	00
	I	0	00	0	00	0	00
	R	6	100	6	100	6	100
FR	S	0	00	0	00	0	00
	I	3	50	0	00	0	00
	R	3	50	6	100	6	100
CN	S	2	33.33	2	33.33	0	00
	I	4	66.67	4	66.67	0	00
	R	0	00	0	00	6	100
NOR	S	6	100	6	100	6	100
	I	0	00	0	00	0	00
	R	0	00	0	00	0	00
CIP	S	6	100	6	100	6	100
	I	0	00	0	00	0	00
	R	0	00	0	00	0	0
ENR	S	6	100	6	100	0	00
	I	0	00	0	00	3	50
	R	0	00	0	00	3	50

PEF: Pefloxacin, AML: Amoxicillin, AMP: Ampicillin, FR: Furazolidone, GN: Gentamycin, NOR: Norfloxacin, CIP: Ciprofloxacin, ENR: Enrofloxacin, R: Resistant, I: Intermediate, S: Sensitive

Results of antibiotic sensitivity tests: The results of antibiotic sensitivity were categorized into Resistant (R), Intermediate (I) and Sensitive (S) based on diameter of zone of inhibition. The results in Table 3 shows that Coagulase positive *Staphylococcus* was sensitive from 33.33 to 66.67% to Pefloxacin, 100% resistant to Amoxicillin and Ampicillin, 50% resistant to Furazolidone and 66.67% sensitive to Gentamycin. But then, this organism was found to be 100% sensitive to Norfloxacin, Ciprofloxacin and Enrofloxacin. *Klebsiella* spp. were

100% sensitive to Pefloxacin and 100% resistant to Amoxicillin, Ampicillin and Furazolidone. In Gentamycin these species were found to be 66.67% sensitive, however, in case of Norfloxacin, Ciprofloxacin and Enrofloxacin these were proved 100% sensitive. In *Pseudomonas* spp. antibiotics Pefloxacin, Amoxicillin Ampicillin, Furazolidone and Gentamycin were 100% resistant. On the other hand, Norfloxacin, Ciprofloxacin were 100% sensitive, however, Norfloxacin was only 50% sensitive. Wayne (2003) isolated Coagulase-positive and negative *Staphylococcus* strains and tested by disc-diffusion, for sensitivity to the following antimicrobials: oxacillin (10 μg), cephazolin (30 μg), ciprofloxacin (5 μg), clavulanic acid+amoxicillin (30 μg), vancomycin (30 μg), azitromycin (15 μg), clindamycin (2 μg), amoxicillin (10 μg), enrofloxacin (5 μg) and ampicillin (10 μg). Cheesbrough (2006) showed *Klebsiella* often produce beta-lactamases and were resistant to ampicillin and some *Klebsiella* strains showed multiple drug resistance. Ndip *et al.* (2005) conducted antimicrobial susceptibility of *Pseudomonas aeruginosa* by the disc diffusion assay. The resistance pattern of cefotaxime, gentamicin and tetracycline was the most common (21.6%) amongst the isolates and there was a significant difference in the susceptibility of isolates to ciprofloxacin (98%), amikacin (90.2%) and netilmicin (80.4%) compared with other drugs used in that study. A study was conducted by Gamal *et al.* (2010) and reported that quinolones, cefipime and linezolide were the most efficient antibiotics in treatment of lower bacterial respiratory tract infections in Egypt. The predominant isolates in that case were *Heamophilus Influnza* (32%), *Streptococcus pneumoniae* (30%), *Moraxella catarrhalis* (14%), *Klebsiella pneumoniae* (10%) and *Chlamydia pneumoniae* (7%). The difference of identified bacteria with the present study might be due to isolation was done here from the clinical cases.

Similarly, Parvez *et al.* (2004) isolated and studied bacterial pathogens: *Staphylococcus aureus*, *Enterobacter aerogenes* and *Escherichia coli* from clinical specimens of hospitalized patients against three antibiotics viz., erythromycin, tetracycline and penicillin G and reported that all the isolates were sensitive to tetracycline and erythromycin.

But then, Sittiwet and Puangpronpitag (2009) reported that *S. aureus* and *E. coli* causing serious nosocomial infection and indiscriminate use of antibiotics showed multi-drug resistant since the bacteria have ability to change the susceptible gene and become resistant to antibiotics. The study highlighted judicious use of antibiotics with proper sensitivity tests following the correct regiment.

It was observed from the present study that *Staphylococcus* spp., *Klebsiella* spp. and *Pseudomonas* spp. were predominant of the bacteria isolated from throat swab of subjects. Percentages of identified bacteria were higher in subjects suffering from respiratory symptoms than apparently healthy subjects. Coagulase Positive *Staphylococcus* (CPS), *Klebsiella* spp. and *Pseudomonas* spp. isolated from respiratory symptoms' subjects were found to be pathogenic. Amoxicillin and ampicillin were not recommended antibiotics to use against *Staphylococcus, Klebsiella* and *Pseudomonas* infection because of their resistance. Ciprofloxacin and Norfloxacin were found to be effective antibiotics to treat Staphylococcal, *Klebsiella* and *Pseudomonas* infections. Isolated *Pseudomonas* spp. showed multidrugs resistant properties. The study provided information about the harboring of potential pathogenic organisms in respiratory systems of apparently healthy subjects and subjects suffering from respiratory symptoms. The pathogenic natures of the isolated organisms were studied that had been evidenced by mouse pathogencity tests. In addition, antibiotics sensitivity guidelines were studied so that the clinicians could treat the patients with appropriate antibiotics.

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