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CULTURE SENSITIVITY PATTERNS OF BACTERIA ASSOCIATED WITH RESPIRATORY ILLNESS IN HUMAN

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ABSTRACT

This study was designed to identify and determine in-vitro antibiotics sensitivity patterns of bacteria associated with respiratory illness of human. A total of 60 throat swab samples were aseptically collected from 3 age groups of 1-20 years (young), 21-40 years (adult) and 41-60 years (old). Isolated bacteria then subjected to determine their in-vitro antibiotic sensitivity patterns. From this study, it was observed that bacterial flora present in the throat swab of human with their percentage of distribution were *Staphylococcus* spp. (40.54%) of which Coagulase positive *Staphylococcus* (32.43%) and Coagulase negative *Staphylococcus* (8.11%), *Klebsiella* spp. (24.325%), *Pseudomonas* spp. (18.92%), *E. coli* (8.11%) and *Bacillus* spp. (8.11%). Percentages of isolated bacteria in different age groups were 29.73% in young, 40.54% in adult and 29.73% in old. Among the isolates Coagulase positive *Staphylococcus*, *Klebsiella* and *Pseudomonas* were the predominant species. Percentages of Coagulase positive *Staphylococcus* spp. were high in age groups of 21-40 years (21.62%) whereas *Klebsiella* spp. were the predominant species of age groups of 1-20 years (10.81%) and 41-60 years (8.11%). *Pseudomonas* spp were the predominant species (8.11%) in age groups of 41-60 years. The isolated Coagulase positive *Staphylococcus* spp and *Klebsiella* spp were resistant to amoxicillin and ampicillin but sensitive to ciprofloxacin, enrofloxacin and norfloxacin. Isolated *Pseudomonas* spp. showed resistant properties against amoxicillin, ampicillin pefloxacin, gentamycin and furazolidone but sensitive to ciprofloxacin and norfloxacin.

Keywords: identification, respiratory illness, human, bacteria, antibiotic sensitivity

INTRODUCTION

It is known that bacteria are the main constituents of the normal flora of the upper respiratory tract. Over 21 genera of the more than 200 species aerobes, anaerobes and facultative anaerobic bacteria colonize on upper respiratory tract (Nadel *et al.,* 1999). Major bacterial pathogens in respiratory infections are *Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli* (LiXia *et al.,* 2008; Watanabe *et al.,* 1995).

Antibiotics are used both in treating human disease and in intensive farming to promote animal growth. Both uses may contribute to the rapid development of antibiotic resistance in bacterial populations (Khachatourians, 1998). One of the most worrisome characteristics of *P. aeruginosa* is low antibiotic susceptibility (Anzai *et al.,* 2000). Being Gram-negative bacteria, most *Pseudomonas spp* are naturally resistant to penicillin and the majority of related beta-lactam antibiotics, but a number are sensitive to piperacillin, imipenem, tobramycin, or ciprofloxacin (Ryan and Ray, 2004).

In our country respiratory illness is very common among different ages of human. Bacteria are the leading cause of respiratory illness. It is very difficult to treat the respiratory illness if proper identification of the causal agent is not performed. On the other hand, multidrugs resistant strains are being developed due to indiscriminate use of antibiotics. Therefore the present work is taken into investigation of resistance bacteria against antibiotics.

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MATERIALS AND METHODS

Collection and transportation of samples

A total of 60 throat swab samples were aseptically collected during febrile and coughing stage in which 20 from each age group. They were divided into 1-20 years (young), 21-40 years (adult) and 41-60 years (old). Immediately after collection the samples were inoculated into nutrient broth. These were then transferred to the Bacteriology Laboratory of Microbiology and Hygiene department, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh using cool box.

Isolation and identification of bacteria

The collected samples were processed as per the procedure of Cheesbrough, 2006. For isolation and identification of bacterial flora, the procedure suggested by Ryan and Ray, 2004. Briefly, the samples were then inoculated into nutrient agar (NA) media and incubated at 37°C for 24 hours. 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. Stock culture was maintained in both agar slant and 20% sterile buffered glycerin.

Antibiotic sensitivity tests

Antibiotic sensitivity tests were performed using disc diffusion test of the method described by Bauer *et al.* (1966). Commercially available antimicrobial discs (Mast Group Ltd., Merseyside, UK) were used for the determination of the drug sensitivity. The concentration of antimicrobial agent per disc was 10µg for gentamycin, 5µg for pefloxacin, 5µg for enrofloxacin, 10µg for norfloxacin, 5µg for ciprofloxacin, 50µg for furazolidone, 25µg for amoxicillin and 10µg for ampicillin. For this purpose, 01-02 ml of freshly growing broth culture were poured on nutrient agar and spread uniformly. Antibiotic discs were placed apart onto the surface of the inoculated plates aseptically with the help of a sterile forceps and incubated at 37°C for 24 hours. After incubation, the plates were examined and the diameters of the zone of bacterial growth inhibition were measured and were interpreted with the standard diameters of NCCLS, (1999) and recorded as sensitive (S), intermediate (I) and resistant (R).

RESULTS AND DISCUSSION

Results of isolation and identification of bacteria from different age groups of human

Upon cultural, morphological and biochemical examinations six bacterial species such as, Coagulase positive and negative *Staphylococcus*, *Bacillus* spp., *Pseudomonas* spp., *Klebsiella* spp. and *Escherichia coli* were identified. In cultural examination Coagulase positive *Staphylococcus* produced gray white or golden yellowish colonies whereas Coagulase negative *Staphylococcus* produced whitish colonies on nutrient agar (NA) plates. Coagulase positive *Staphylococcus* produced beta-hemolysis on Blood agar (BA) plates however Coagulase negative *Staphylococcus* could not produce hemolysis on BA plates. *Bacillus* produced thick grayish white or cream coloured colonies on NA plates and beta-hemolysis on BA plates. *Pseudomonas* produced a blue-green pigment (pyocyanin) on the NA plates; large, flat, spreading and pigmented colonies with hemolysis on BA plates; pale coloured colonies on MacConkey (MC) agar and Salmonella-Shigella (SS) agar plates and pigmented colonies on Eosime Methylene Blue (EMB) agar plates. These were similar findings reported earlier by Levinson (2006).

Klebsiella produced mucoid, circular, white to grayish white colonies on NA plates; pink mucoid colonies on MC agar (Fig.1) and SS agar plates; purple mucoid colonies on EMB agar plates and hemolysis on BA plates. However *E. coli* produced smooth, circular, white to grayish white colonies on NA plates; rose pink colonies on MC and SS agar plates, moist circular colonies with dark centered yellow green metallic sheen on EMB agar plates and no hemolysis on BA plates. These types of colonies were reported in *Klebsiella* previously by Baron (1996). He reported mucoid colonies on NA, EMB, MC and SS agar plates.

Triple sugar iron (TSI) agar slant became yellowish with accumulation of gas in case of *Klebsiella* (Fig.2) and *Escherichia* whereas both the slant and butt became pinkish in case of *Pseudomonas* (Fig.2). Ryan and

Ray (2004) also reported yellowish slant and butt in case of *Klebsiella* and *Escherichia,* but no colour change incase of *Pseudomonas*. Individual colonies from pure culture with Gram's revealed Gram positive cocci arranged singly or in cluster, *Bacillus* spp. revealed Gram positive large spore forming rods arranged in chain. *Pseudomonas* (Fig.3), *Klebsiella* and *Escherichia* revealed Gram negative rods. These findings were supported by Cheesbrough (2006).

The prevalence of bacterial affection in the throat of human origin is presented in Table 1. Percentages of Coagulase positive *Staphylococcus* spp. were high (21.62%) in 21-40 years age groups and *Klebsiella* spp. were in age groups of 1-20 years (10.81%) and 41-60 years (8.11%). *Pseudomonas* spp. were the predominant species in age groups of 41-60 years (8.11%). Similar studies were conducted by a group of scientist (Kabra *et al.,* 2004; Dedeic *et al.,* 2007 and Berkovitch, 2009). Research conducted by Todar (2008) showed the predominant bacterial flora of respiratory tract in human are *Staphylococcus epidermidis*, *Staphylococcus aureus, Streptococcus* spp., *Klebsiella* spp.,

Table 1. Prevalence of bacterial flora of human respiratory tract based on age group

Name of isolates	Number of isolates			Total (%)
	1-20 years (Young) (%)	21-40 years (Adult) (%)	41-60 years (Old) (%)	
Coagulase positive *Staphylococcus*	4 (5.41)	16 (21.62)	4 (5.41)	24 (32.43)
Coagulase negative *Staphylococcus*	2 (2.70)	2 (2.70)	2 (2.70)	6 (8.11)
***Klebsiella* spp.**	8 (10.81)	4 (5.41)	6 (8.11)	18 (24.33)
***Pseudomonas* spp.**	4 (5.41)	4 (5.41)	6 (8.11)	14 (18.92)
Escherichia coli	2 (2.70)	2 (2.70)	2 (2.70)	6 (8.11)
***Bacillus* spp.**	2 (2.70)	2 (2.70)	2 (2.70)	6 (8.11)
Total	22 (29.73)	30 (40.54)	22 (29.73)	74 (100)
	74 (100)			

Pseudomonas spp., *Proteus* spp., *E. coli.* The observation result of sensitivity against antibiotics discs were categorized into resistant (R), intermediate (I) and sensitive (S) based on diameter of zone of inhibition. The results are given in Table 2. It revealed that 30% Coagulase positive *Staphylococcus* (CPS) is sensitive and 70% gave intermediate reaction to pefloxacin. On the other hand, 40% is sensitive and 60% is intermediate sensitive to gentamycin. Hundred percent (100%) of the isolates were resistant to amoxicillin and ampicillin, 50% sensitivity and 50% intermediate reaction to furazolidone, 100% sensitivity to norfloxacin, ciprofloxacin and enrofloxacin (Table 2, Fig.4). 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 susceptibility of isolates to ciprofloxacin (98%), amikacin (90.2%) and netilmicin (80.4%). Among the bacteria isolated from throat swab of human *Staphylococcus* spp., *Klebsiella* spp. and *Pseudomonas* spp. were the predominant species. Percentages of identified bacteria were comperatively higher in adult than young and old ages. Percentages of Coagulase positive *Staphylococcus* spp. were high in adult whereas *Klebsiella* spp. were the predominant species of both

Table 2. Antibiotic sensitivity tests of bacteria isolated from human throat

Name of antibiotics	Sensitivity and Resistant patterns	Coagulase positive *Staphylococcus* (%)	*Klebsiella* spp. (%)	*Pseudomonas* spp. (%)
Pefloxacin (PEF)	Sensitive (S)	30	100	0
	Intermediate (I)	70	0	0
	Resistant (R)	0	0	100
Amoxicillin (AML)	Sensitive (S)	0	0	0
	Intermediate (I)	0	0	0
	Resistant (R)	100	100	100
Ampicillin (AMP)	Sensitive (S)	0	0	0
	Intermediate (I)	0	0	0
	Resistant (R)	100	100	100
Furazolidone (FR)	Sensitive (S)	0	0	0
	Intermediate (I)	50	0	0
	Resistant (R)	50	100	100
Gentamycin (CN)	Sensitive (S)	40	40	0
	Intermediate (I)	60	60	0
	Resistant (R)	00	0	100
Norfloxacin (NOR)	Sensitive (S)	100	100	100
	Intermediate (I)	0	0	0
	Resistant (R)	0	0	0
Ciprofloxacin (CIP)	Sensitive (S)	100	100	100
	Intermediate (I)	0	0	0
	Resistant (R)	00	0	0
Enrofloxacin (ENR)	Sensitive (S)	100	100	0
	Intermediate (I)	0	0	50
	Resistant (R)	0	0	50

young and old and *Pseudomonas* spp. in old. Amoxicillin and ampicillin would not be good to use against *Staphylococcus*, *Klebsiella* and *Pseudomonas* infection because of their resistance. Ciprofloxacin and norfloxacin were used as effective antibiotics to treat Staphylococcal, *Klebsiella* and *Pseudomonas* infections. On the other hand, enrofloxacin is also recommended for Staphylococcal and *Klebsiella* infection where as pefloxacin only for *Klebsiella* infection. Isolated *Pseudomonas* spp. also showed resistant properties against pefloxacin, gentamycin and furazolidone.

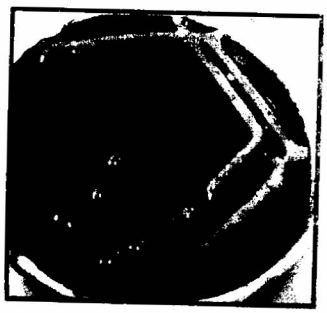

Fig. 1. A representative culture of *Klebsiella* species in MacConkey agar plate showing pink mucoid colonies.

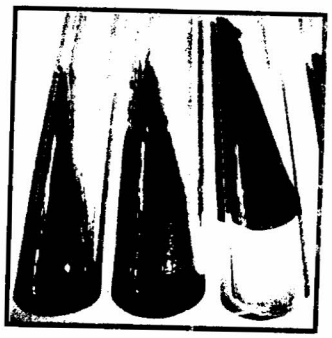

Fig. 2. Plate showing agar slant cultures of isolated bacteria (left one is control, middle one is *Pseudomonas* species and right one is *Klebsiella* species).

Fig. 3. A representative stained slide of *Pseudomonas* species isolated from human throat.

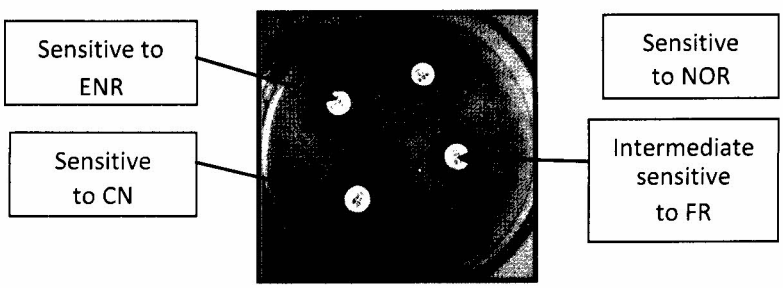

Fig. 4. Plate showing sensitivity and resistant pattern of isolated Coagulase positive *Staphylococcus* against Enrofloxacin (ENR), Gentamycin (CN), Norfloxacin (NOR) and Furazolidone (FR).

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