Isolation and Identification of Microorganism from Sputum of Chronic Obstructive Pulmonary Disease Patients

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Abstract:

Bacterial respiratory infections especially in patients with chronic obstructive pulmonary disease (COPD), is still continue as common health problem especially in developing countries with high morbidity and mortality often due to respiratory failure. Isolation and identification of common bacterial pathogens among COPD patients and to determine the drug sensitivity and resistant pattern of isolated pathogens was my research objective. It was a cross sectional study and study was conducted among purposively selected 250 samples. In this study, bacterial pathogens were isolated from sputum. In all cases Pseudomonas spp. (38.55%) was the most common frequent isolates followed by Escherichia coli (16.87%), Klebsiella spp. (34.93%), staphylococcus aureus (3.61%), streptococcus spp. (3.61%) and Acinetobacter spp. (2.40%). Antimicrobial susceptibility test was done

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and cotriamoxazole, aztreonam, cefuroxime, levofloxacin, ciprofloxacin, amikacin, imepenem and gentamicin were found effective in majority of the isolates. Increasing resistance against these drugs is alarming. Therefore, rational antibiotic therapy of bacterial respiratory infections especially in COPD exacerbation must be chosen on culture and sensitivity report.

Key words: Identification of Microorganism, Chronic Obstructive Pulmonary Disease

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality across the world. The World Health Organization, in 1998, ranked COPD as fifth in the list leading causes of mortality and disease burden worldwide. In Southeast Asia, it ranked number 11 in the same year.[1] It is projected that by the year 2020, COPD will rank fourth as the leading cause of disease burden in developing regions, surpassing cerebrovascular disease.[2] COPD is a slowly progressive airways disease characterized by a gradual loss of lung function due to airflow obstruction brought about by chronic bronchitis or emphysema. Acute exacerbations of COPD represent a major health burden. It is both an economic and social burden because of the propensity for readmissions associated with exacerbations and the resulting transient or permanent decrease in quality of life. The etiology of exacerbations varies and this includes increased airflow obstruction, mucus plugging and retention, fluid retention, as well as bacterial infections. Infection is usually considered the main and most common cause of acute exacerbation; however, the exact role of bacterial infections is not well defined because of the conflicting data on the role of the tracheo bronchial flora and the usefulness of antibiotic therapy.[3] COPD patients have evidence of long standing bacterial colonization in the lower respiratory tract during periods of remission as well as during exacerbations.

Organisms that have the potential to be pathogenic can be recovered from the respiratory tract secretions of virtually all COPD patients at some time during the course of their disease. The present study is an attempt to explore isolation and identification of microorganism from sputum of chronic obstructive pulmonary disease (COPD) patients.

Materials and Methods

Study site: This was a cross sectional criterion standard validation study conducted at the National Institute of Diseases of the Chest and Hospital (NIDCH), Mohakhali, Dhaka-1212 in outdoor patients and in door patients. The laboratory works were carried out in the Department of pathology and Microbiology at NIDCH.

Study period: This study was carried out during the time period December 2010 to May 2011.

Sample size: A total of 250 sputum samples were studied. A cross-sectional, epidemiologic study was conducted to identify isolated bacteria in the sputum of exacerbated COPD patients and to relate them to the degree of functional impairment.

Collection of Specimen: Sputum was collected in a sterile disposable wide mouthed, screw-capped plastic container of about 100 ml capacities early in the morning preferably before taking any antibiotics. The patient was first asked to wash the mouth with warm water and then to spit out cough in to the container. All the specimen were then sent to the laboratory within 2(two) hour after collection for processing.

Screening of Sputum

Naked eye examination: Purulent sputum containing yellow or green opaque material as well clear mucoid secretion were included in this study. Those which were clear and watery were discarded.

Microscopic Examination: The equal volume of sputum was mixed with equal volume of sterile solution of sputolyxin (oxoid) and mixed gently on a rotator and then incubated at 37 ^{o}C for 15 minutes. A gram smear was then prepared with standard procedure. If the smear shows less then 10 polymorphs to every squamas epithelial cells the specimen was then discarded.

Study Population: Patients from both sexes and all ages who were attended in NIDC Hospital and had COPD and symptoms of acute exacerbation were screened for Diagnosis.

Microbiological Study of sputum samples
At first, sputum samples were obtained from patient attending out-patient department or in the emergency department. Samples were collected in a sterile vial and sent within 2h into a laboratory.

Microscopic Examination
A Gram's stain of sputum in the area of maximal purulence was examined for polymorphonuclear leukocytes and epithelial cells. The following criteria, based on the criteria of were applied in order for a sputum sample to be deemed acceptable for analysis: a microbiological study done by using a low-magnification lens (10x) reveals samples with < 10 epithelial cells and > 25 leukocytes per field were accepted for further analysis followed by criteria based on the criteria of *Murray and Washington and Heineman et al.* In cases with more than one sputum sample the following criteria were used (in the

order that is listed) to select a sample: quantity of bacterial growth (more was better than less) and quality of sputum (the greater the number of leukocytes and the fewer the number of epithelial cells the better).

Isolation of Microorganisms

Selected sputa were processed microbiologically for quantitative study following accepted laboratory methods. Using the microbiological loop, 0.01 ml sputa were spreading in the culture media: Incubation was carried out at $35 \pm 2°C$ in aerobic conditions. A first reading was taken after 24 h, and a second final one was taken after 48 h of culture. Following microbiological media were used for isolation of different pathogenic bacteria from the samples:

1. **MacConkey agar (MAC):** MacConkey agar was used for the isolation of gram-negative bacteria and used for differentiate between lactose fermenter & non-lactose fermenter.
2. **Cetrimide agar:** used for the isolation of gram-negative bacteria, *Pseudomonas aeruginosa,* which show a characteristics blue-green & yellow-green color respectively.
3. **Mannitol salt agar (MSA):** Selective for gram-positive organisms.
4. **Nutrient agar (NA):** Contain all the elements that most bacteria need for growth and are non-selective, so they are used for the general cultivation and maintenance of bacteria kept in laboratory culture collections.

Identification of bacterial isolates

Both primary and confirmative identification of bacteria was performed. According to 'Biochemical Test for Identification of Medical Bacteria' by Zean F. MacFabdin (1980) and 'Microbiological Laboratory Manual' by G. Cappuccino and

Natalie Fhernan (1983), several biochemical tests were performed to identify the bacteria of interest.

Data processing: After collection, data were checked thoroughly for consistency and completeness. Data were checked after collection of data to exclude any error or inconsistency.

Statistic Analysis: All analysis was done by appropriate statistical methods using SPSS software for Windows version 11.5.

Ethical Issue: All ethical issues, which were related to the research involved with human subjects, were followed according to the guideline of ethical review committee.

Result

Primary identification of bacteria (Figure 1)
Primary identification of bacteria was performed based on the selectivity of media, change of media after growth, morphological characteristics of the colonies and microscopic characteristics. Morphological characteristics including size, shape, surface, texture, edge, elevation, color, opacity etc from different types of colonies in different culture media were observed and recorded. Identification of bacterial isolates was carried out according to Bergey's manual.
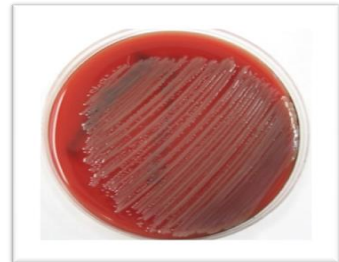
Colony morphology on MacConkey and Blood agar plate
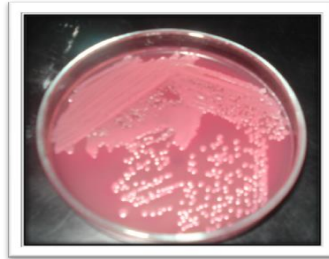
Growth of Escherichia coli on MacConkey agar plate

Growth of Klebsiella spp. on MacConkey agar plate

Pseudomonas spp. on blood agar plate

Biochemical tests for identification of bacterial isolates (Table 1)

After growth on MacConkey and Blood agar plates, the isolates were identified by biochemical tests as Kligler Iron Agar (KIA) test, Motility Indole Urea (MIU) test, Citrate test and Oxidase test. They were confirmed as respective organisms according to Biochemical Test. For Identification of Medical Bacteria by Zean F. MacFabdin (1980) and Microbiology Laboratory Manual by G. Cappuccino and Natalie Fhernan (1983). Examples of biochemical reaction of some gram-negative bacteria are given in the following table

Isolates	KIA[®]			MIU[€]			Citrate test	Oxidase test
	Slant	Butt	H_2S	Mot[∞]	Ind[η]	Urea		
Escherichia coli	K/A	AG	-	+/-	+	-	-	-
Klebsiella	A	AG	-	-	-	+/-	+	-

spp.								
Pseudomonas spp.	No change	No change	-	+/-	-	+/-	+/-	+
Acinetobacter spp.	No change	No change	-	-	No change	-	+/-	-

¥Symbols: K: Alkaline reaction, A: Acidic reaction, AG: Acidic reaction & gas, +:Positive, - : Negative, ® Kligler Iron agar test, € Motility Indole Urea test, ∞ Motility,   η Indole

Frequency of pathogens isolated from sputum samples (Figure 2)

A total of 83 bacterial pathogens were isolated. These include 38.55% Pseudomonas spp., 16.87% Escherichia coli, 34.93% Klebsiella spp., 3.61% Staphylococcus aureus, 2.40% Acinetobacter spp. and 3.61% Streptococcus spp. No bacterial pathogens were isolated in 66.8 % cases.
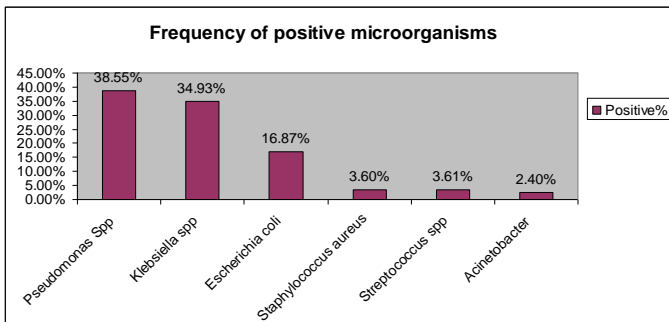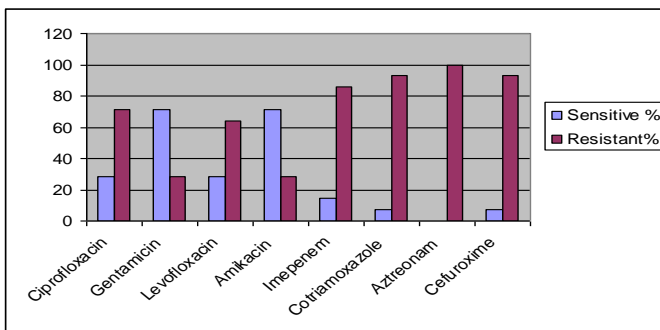

Antimicrobial susceptibility pattern of Pseudomonas spp. (Figure 3)

Figure shows the antimicrobial susceptibility pattern of Pseudomonas spp. Pseudomonas spp. were sensitive to gentamicin (81.2%) followed by levofloxacin (81.2%), ciprofloxacin (78.1%), amikacin (62.5%) and cotriamoxazole (34.37%r). The isolates were resistant to imipenem, aztreonam and cufuroxim (96.87%, 93.75% and 100%, respectively).
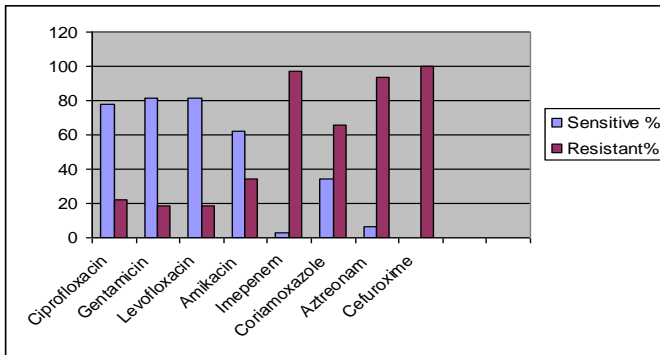
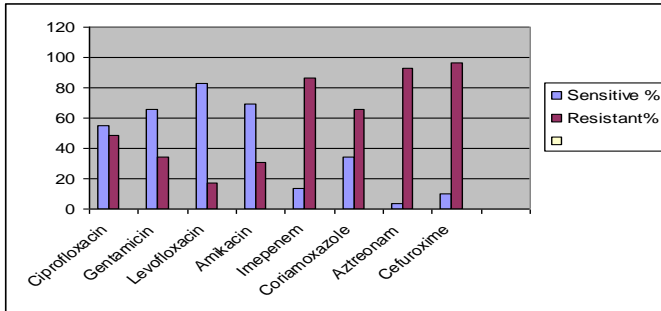## Antimicrobial susceptibility pattern of Escherichia coli (Figure 4)

Figure indicates the antimicrobial susceptibility pattern of Escherichia coli. E. coli isolates were sensitive to gentamicin (71.42%) followed by amikacin (71.42%), ciprofloxacin (28.57%) and levofloxacin (28.57%). Isolates were resistant to imepenem, cotriamoxazole and cefuroxime (85.71%, 92.85% and 92.85%, respectively).

Antimicrobial susceptibility pattern of Klebsiella spp. (Figure 5)

Figure shows the antimicrobial susceptibility pattern of Klebsiella spp. Isolates were sensitive to levofloxacin (82.75%) followed by amikacin (68.96%), gentamicin (65.51%), ciprofloxacin (55.17%) and cotriamoxazole (34.48%). The

isolates were resistant to cefuroxime, aztreonam and imepenem
(96.55%, 93.10% and 86.20%, respectively).

Antimicrobial susceptibility pattern of Staphylococcus aureus (Figure 6)

Figure shows the antimicrobial susceptibility pattern of
Staphylococcus aureus. Isolates were sensitive to levofloxacin
(100%) followed by gentamicin (100%), ciprofloxacin (66.66%)
and cotriamoxazole (33.33%). The isolates were completely
resistant to amikacin, imepenem, aztreonam and cefuroxime
(100%).
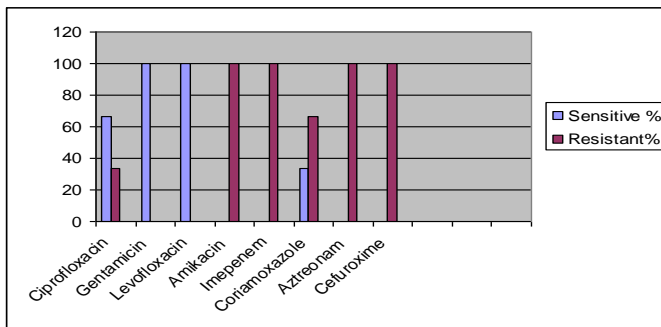
Discussion

Respiratory tract is the major connection between the interior
of the body and outside environment and therefore, more
vulnerable to infection. Smoking, bacterial infections and
inhalation of dust appears to be responsible for the

exacerbation of chronic obstructive pulmonary diseases.[4] Suppurative pyogenic infection of the chest as one of the major cause of morbidity and mortality especially in elderly patient and NIDCH is an unique institute of the country where all chronic and elderly patient with respiratory infections are admitted. In this study respiratory specimen namely sputum were analyzed. 33.2% sputum specimens were cultured with bacterial pathogens. Among them, in all cases Pseudomonas spp appeared as major pathogen followed by Klebsiella spp, Pseudomonas spp, Escherichia coli, Staphylococcus aureus, Streptococcus spp, and Acinetobacter spp., but there is no such data to compare our result especially on such large scale hospital based study. The species differ in this study because majority of patients were in chronic cases and in most cases, the pathogens were secondary invader.[5] The antibiogram of this study correlated with other study[6], ciprofloxacin, gentamicin, levofloxacin, amikacin still remain as effective antimicrobials for the treatment of chronic obstructive pulmonary disease patients. As there is diversity of pathogen, and gradually increasing resistant strains, the treatment of COPD patients should given on the basis of a good culture and sensitivity report.[7]

Conclusion

Klebsiella spp and pseudomonas spp are the most common sputum pathogens in hospitalized patients with COPD(chronic obstructive pulmonary disease). in coclusion, good culture facilities are well, as appropriate hospital infective control program can minimize chest infections in consequence morbidity and mortality of the patient. Antimicrobials resistance monitoring should be continued to see the changing pattern of the isolates to give appropriate antibiotic therapy.

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