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Emerging Drug Resistance in Sputum-Derived Gram Negative Pathogens

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ABSTRACT
In recent decades, there has been a growing interest about the role of gram negative bacteria in sputum samples. Identifying gram negative bacteria and in particular resistant gram negative bacteria is of paramount importance in patients with higher clinical severity and unfavorable outcomes. The emerging pathogens were isolated by culture and antibiotic susceptibility testing. To classify the various Gram-negative bacteria, biochemical tests namely indole, citrate utilization, triple sugar iron (TSI), urease and oxidase were performed. Sputum samples of patients were collected and cultured for detection of microorganisms and further subjected to Antibiotic sensitivity and biochemical tests in the Department of Microbiology, KPC Medical College and Hospital, Jadavpur, Kolkata. During the study period 1 year, a total of 200 sputum samples were received for bacterial culture. In our study, 143 were gram negative bacteria and rest of the samples were either gram positive or environmental isolates. Out of these, 65 samples were found to be multidrug resistant Gram negative bacteria. In this study, four Gram-negative bacteria namely *Klebsiella pneumoniae*, *Pseudomonas* sp., *E. coli* and *Proteus* sp. were isolated from sputum samples of patients.

INTRODUCTION

One test to look for microorganisms in the respiratory system and lungs is a sputum culture. Phlegm, or sputum, is a viscous mucus produced by the lungs. Immune system cells found in sputum aid in the defense against bacteria, fungus and other foreign objects in our lungs and airways. Sputum's thickness aids in capturing the foreign object. This enables the cilia, or microscopic hairs, in the airways to force it through the lips and out through coughing. Sputum can come in a variety of hues. The colors can indicate if a chronic condition has worsened or what kind of infection a person may have.

A sputum culture is most often used to:

- Find and diagnose bacteria or fungi that may be causing an infection in the lungs or airways
- See if a chronic illness of the lungs has worsened
- See if treatment for an infection is working[1]

Klebsiella sp., *Haemophilus* sp., *Pseudomonas* sp., *Legionella* sp., *Mycoplasma* sp., *Staphylococcus* sp., *Streptococcus* sp. and other common microorganisms have been recovered from sputum cultures. These are a few significant gram-negative bacteria that are frequently connected to the respiratory system. *Legionella pneumophila* is mostly found in ambient water sources, although *H. influenzae* and *Bordetella pertussis* are two examples of Gram-negative rods that produce significant levels of illness and death. *Acinetobacter baumannii* colonizes the skin and upper respiratory tract in addition to being present in ambient water sources.

In contrast to gram-positive bacteria, gram-negative bacteria lose the crystal violet stain that is utilized in the Gram staining technique for bacterial differentiation[2]. Their cell envelope, which is made up of an exterior and an inner (cytoplasmic) membrane sandwiched between a thin peptidoglycan cell wall, is what makes them unique. Gram staining was once used to classify species at the subdivision of bacteria and is a quick diagnostic method for categorization[3]. One of the several unique characteristics of gram-negative bacteria is the structure of the bacterial outer membrane. The outer leaflet of this membrane contains lipopolysaccharide (LPS), whose lipid A portion acts as an endotoxin. If gram-negative bacteria enter the circulatory system, LPS can trigger an innate immune response, activating the immune system and producing cytokines (hormonal regulators).

MATERIALS AND METHODS

Sputum samples of patients were collected and cultured for detection of microorganisms and further subjected to Antibiotic sensitivity and biochemical tests in the Department of Microbiology, KPC Medical College and Hospital and Jadavpur.

Study period: September 2022-August 2023.

Procedure:

Sample collection: Samples were collected in sterile plastic containers and transferred to the microbiology lab of KPC Medical College.

Sample size: 200

Gram staining was performed on the collected samples.

Sample processing: One loopful of freshly collected Sputum samples were streaked on MacConkey Agar and Blood agar plates and marked as per the patient ID. The plates were incubated at 37EC for 24 hrs.

The next day Biochemical Tests and antibiotic sensitivity tests (AST) were performed on the colonies isolated from DAY 1 by Disc diffusion method (Kirby Bauer method).

RESULTS

In our study, 10 patients were found to be Intermediately Sensitive to Trimethoprim/ sulfamethoxazole and 4-5 patients were found to be Resistance to Ceftazidine (Table 1).

DISCUSSION

Sputum analysis can thus be referred to as a simple and effective method to detect respiratory diseases. It provides the most critical information about a patient's condition, its severity and helps to choose the right treatment regimen. The studies shown above implies that the microorganisms isolated from the sputum of these patients are multidrug resistant to three or more classes of antimicrobial drugs.

MDR bacteria adopt a variety of modifications to withstand the environmental harm that antibiotics inflict. Through a mechanism known as horizontal gene transfer, resistant bacteria can exchange genetic material that encodes resistance to the naïve population, allowing them to share these resistance traits[4].

Antibiotic inactivation: bacteria create proteins that can prevent damage caused by antibiotics, they can do this in two ways. First, inactivating or modifying the antibiotic so that it can no longer interact with its target. Second, degrading the antibiotic directly.

Multidrug efflux pumps: The use of transporter proteins to expel the antibiotic.

Table 1: Intermediately sensitive vs resistance

Antibiotics	Diameter of Zone of Inhibition in mm (after deducting disc diameter = 6 mm)	Resistance pattern
Amoxicillin+clavulanic acid	0	Resistant
Amikacin	0	Resistant
Aztreonam	0	Resistant
Cefotaxime	0	Resistant
Trimethoprim/sulfamethoxazole	10	Intermediately sensitive
Ceftriaxone	0	Resistant
Cefepime	0	Resistant
Chloramphenicol	7	Resistant
Ceftazidine	4-5	Resistant
Ciprofloxacin	0	Resistant
Cefuroxime	0	Resistant
Doxycycline	0	Resistant
Gentamicin	0	Resistant
Imipenem	0	Resistant
Levofloxacin	0	Resistant
Meropenem	0	Resistant
Polymyxin B	9	Intermediately sensitive
Tetracycline	0	Resistant
Tobramycin	0	Resistant
Tigecycline	3-4	Resistant

Modification of target sites: Mutating or modifying elements of the bacteria structure to prevent interaction with the antibiotic.

C **Structural modifications:** Mutating or modifying global elements of cell to adapt to Antibiotic (Such as increased acid tolerance to an acidic antimicrobial)[5].

However, the concentration of antibiotics used in this study might have been insufficient to be effective against the pathogens isolated from the sputum samples. It is suggested that increasing the concentration of antibiotics might provide better results.

Cotrimoxazole and Polymyxin B were the antibiotics discovered in this investigation that were susceptible to the Klebsiella group of pathogens. Trimethoprim and sulfamethoxazole together form cotrimoxazole. Sulfamethoxazole competes with para-aminobenzoic acid (PABA) to prevent bacterial synthesis of dihydrofolic acid, while trimethoprim binds to and reversibly inhibits dihydrofolate reductase, an enzyme that is necessary to produce tetrahydrofolic acid from dihydrofolic acid. Thus, cotrimoxazole blocks two consecutive steps in the biosynthesis of nucleic acids and proteins essential to the bacteria. Polymyxin B disrupts the outer cell membrane of Gram-negative bacteria, binds and neutralizes lipopolysaccharide and inhibits respiration of the Gram- negative bacterial cells[6].

The medications Ciprofloxacin, Levofloxacin, Ceftazidine, Colistin, Piperacillin/Tazobactam, Imipenem, Meropenem and Tetracycline were shown to be susceptible to the Pseudomonas group of pathogens. Fluoroquinolones such as levofloxacin and ciprofloxacin target DNA gyrase and topoisomerase IV in different bacteria with differing degrees of efficiency. By inhibiting these enzymes' ability to control supercoiling inside the cell, the drugs impair DNA replication at lower concentrations and cause cell death at lethal concentrations. Penicillin-binding protein 3 (PBP3) and other enzymes involved in cell wall production are the main targets of ceftazidime's bactericidal effects[7]. Colistin penetrates into and disrupts the bacterial cell membrane. It interacts with the bacterial cytoplasmic membrane, changing its permeability. This effect is bactericidal. Piperacillin kills bacteria by inhibiting the synthesis of bacterial cell walls. It binds preferentially to specific penicillin-binding proteins (PBPs) located inside bacterial cell walls. Tazobactam inhibits the action of bacterial beta-lactamase producing organisms, which are normally resistant to beta- lactam antibiotics. Thus, Tazobactam broadens the spectrum of piperacillin[8]. Imipenem and Meropenem are Carbapenem antibiotics which kill bacteria by binding to penicillin-binding proteins, thus inhibiting bacterial cell wall synthesis[9].

Tetracycline and imipenem were the medications that worked well against *Escherichia coli*. Tetracycline stops translation, which stops bacterial growth. It attaches itself to the 30S ribosomal subunit and stops the amino-acyl tRNA from attaching to the ribosome's A site. Additionally, it partially attaches to the 50S ribosomal subunit. In nature, this binding is reversible[10]. The antibiotics effective against *Proteus* group of organisms were meropenem, imipenem and cotrimoxazole. The mechanism of action has been mentioned above.

CONCLUSION

Four Gram-negative bacteria were identified from patient sputum samples in this study: *Klebsiella* sp., *Pseudomonas* sp., *E. coli* and *Proteus* sp. Tests for antibiotic sensitivity and biochemistry were conducted on the isolated organisms. It was discovered that

the microbes were resistant to many drugs. Therefore, further research is necessary to stop the aforementioned bacteria's resistant forms from forming and causing terrible respiratory illnesses.

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