A Study of Bacteriological Profile of Bacterial Isolates in Sputum Samples in Azadi Teaching Hospital in Duhok City

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Abstract

The objective of this current study is to examine the bacteriological profile of microorganisms isolated in sputum samples collected from the patients in the Azadi Teaching Hospital in Duhok City. The current cross-sectional observational study was carried out in the Laboratory of Microbiology, Department of the Basic Science, College of Nursing at the University of Duhok, Kurdistan region, Iraq. A total of 150 patients with clinically verified bacterial infections were included in the study using a gram-staining and routine sampling procedure. Microscopic examination of the gram-stained sputum smears was done to assess the presence of epithelial cells, pus cells, or organisms. Consequently, 83 bacterial samples yielded positive results that indicated the presence of bacterial growth, while the remaining samples 67 did not exhibit any bacterial growth or normal flora. On assessing the organisms isolated from the study subjects, it was observed that out of 150 processed sputum samples, 44.7% (n=67) were non-bacterial samples and 55.3% (n=83) were pathogenic bacterial samples. It was seen that the most common isolated organism was Klebsiella pneumoniae in (44.6%; 37/83), followed by Pseudomonas aeruginosa in (35%; 29/83), and then Escherichia coli in (20.4%; 17/83), were the most frequently isolated microorganisms from sputum samples of patients with upper or lower respiratory infections. The current study comes to the conclusion that in order to obtain lower respiratory tract infection samples from subjects, high-quality sputum must be obtained, and initial sputum screening should be carried out.

Introduction

Significant morbidity and mortality are caused by upper and lower respiratory tract infections, particularly in older patients, those with a history of lung disease, and patients with immune system suppression [1]. The most common sample used to diagnose lower respiratory tract infections is sputum because it is easy to collect and the procedure is non-invasive [2]. For a practical and cost-effective diagnosis of lower respiratory tract infections, sputum culture is essential. However, the chance of saliva and oral flora contamination during sample collection compromises the sample’s credibility. It also avoids wasting time and resources by improperly processing contaminated samples that are not useful for patient care [3]. To ascertain whether a sputum sample is purulent, a standard gram stain must be performed prior to inoculating it into the culture media. Sputum samples are examined under a microscope to ensure that patients with lower respiratory tract infections do not receive the incorrect culture [4]. Lower respiratory tract infections, or LRTIs, are common in the general population, though they primarily affect the elderly, those with long-term illnesses, and those with compromised immune systems. A number of organizations, including the Infectious Disease Society of America and the British Thoracic Society (BTS), have developed the main treatment guidelines for LRTIs (IDSA) [5, 6]. In order to treat LRTIs, it is crucial to identify the infectious organisms that cause them [6]. Infection can occur because of infectious agents including bacteria, viruses, fungi, and protozoa. These agents encompass a wide range of organism such as [7] Streptococcus pneumonia, Staphylococcus aureus, Hemophilus influenza, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Proteus species, Histoplasma capsulatum, and Candida albicans. Most common organism are Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus. A high-quality sputum
sample requires patient understanding and healthcare professional education [8]. It is likely not cost-effective to culture high-quality sputum samples obtained from patients receiving antibiotic treatment [2]. When treating bacterial illnesses, bacteria may become resistant to one or more antibiotics [8]. Among the ways that organisms exhibit antibiotic resistance are drug inactivation or modification, target site alteration, metabolic route alteration, and decreased drug accumulation. This investigation aims to identify the causative bacteria from various sputum samples.

**Materials and Methods**

**Period of the study**
The period of this study was from September 2023 to November 2023.

**Sample Collection**
Gather the materials in a wide-mouthed, screw-capped plastic container with a capacity of no more than 100 ml for disposal (Figure 1). Provide the sample to the laboratory as soon as you can, ideally in two hours, since infections particularly those caused by sensitive bacteria may disappear if you wait too long [9].

![Figure 1: Sample (Sputum) Container](image)

**Sample Processing**

**Gram stain:** For the sputum samples, the standard loop technique and direct gram stain were employed. On Blood Agar, MacConkey Agar, and Nutrient Agar, sputum was cultured. Plates were incubated at 37 °C for the entire night. Microscope analysis was used to interpret the results of gram-stained smears.

**Biochemical Tests:** Numerous tests were carried out, such as the Triple Sugar Iron (TSI), sugar fermentation, oxidase test, catalase test, and INViC test (Indole, Methyl Red, Voges-Proskauer, and Citrate).

**Result and Discussion**
These biochemical tests, along with colony morphology, pigment production, lactose fermentation (and non-lactose fermentation), Gram staining, oxidase test, and catalase test, were all used to identify the bacteria. 150 (100%) sputum samples were analyzed for our study; 83 (55.3%) of the samples had positive results, meaning that bacteria were growing, and the other samples 67 (44.7%) either showed no bacterial growth at all or normal flora (Table 1). The most frequently isolated organism from the samples was *Klebsiella pneumoniae* (44.6%; 37/83), followed by *Pseudomonas aeruginosa* (35%; 29/83) and *Escherichia coli* (20.4%; 17/83) (Table 2).

<table>
<thead>
<tr>
<th>Bacterial name</th>
<th>No. (n=83)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>37</td>
<td>44.6</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>29</td>
<td>35</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>17</td>
<td>20.4</td>
</tr>
</tbody>
</table>

One easy-to-use and reliable method for identifying bacterial infections is sputum culture. The goal of the current investigation was to examine the gram staining and sputum culture of participants suffering from lower respiratory tract infections. The study comprised 150 sputum samples that were examined under a microscope to determine whether epithelial cells, pus cells, or microorganisms were present after gram staining.

The majority of the samples in this investigation were mucoid sputum, which was followed by purulent and mucopurulent sputum. Most purulent/mucopurulent sputum samples produced positive cultures when compared to mucoid sputum samples. 18 (35.86%) of the 150 sputum samples had bacteria isolated from them. Of the organisms isolated from sputum culture in our study, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* were the most frequently isolated isolates. This is comparable to a study conducted in 2023 by Gupta and colleagues, which also revealed *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* were the most frequently isolated microorganisms from sputum
samples [8]. Additionally, the results of this study closely align with those of a prior investigation that found that *Klebsiella pneumoniae* was the most frequently isolated organism, isolated from 28.40% (n=25) samples, followed by *Pseudomonas aeruginosa* in 14.77% (n=13) samples and *Escherichia coli* in 12.5% (n=11) samples [10]. These outcomes concurred with those of Lloveras et al. [11] and Ziyade and Yagci [12], whose investigations revealed that comparable microorganisms had been extracted from the expectorated sputum of their research participants. Variations in sample collection techniques, timing, mode of transportation, and sample processing may account for variations in the frequency of bacterial isolation in sputum across studies.

**Conclusion**

Within its limitations, the present study concludes that in subjects with Lower respiratory tract infections, good-quality sputum must be obtained and initial screening of the sputum should be done to obtain it. The present study had a few limitations including a small sample size, shorter monitoring period, and geographical area biases. Hence, more longitudinal studies with larger sample sizes and longer monitoring periods will help reach a definitive conclusion.

**References**


