



## Research Article

# A Study of Bacteriological Profile and Antimicrobial Susceptibility Pattern of Bacterial Isolates in Sputum Samples in Tertiary care hospital

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## Abstract

**Aim:** The study's objective is to examine the bacteriological profile and antibiotic susceptibility pattern of isolated organisms in sputum samples in tertiary care hospitals.

**Material and method:** In Lucknow's Era's Lucknow Medical College and Hospital, the microbiology department conducted the current cross-sectional study. Using a regular sampling technique, a total of 1503 patients with clinically confirmed bacterial illnesses were included in the study. As a consequence, 108 bacterial samples produced positive results suggesting bacterial growth, whereas the other samples showed no bacterial growth or normal flora. The Bartlett's procedures were used to assess the sputum's quality.

**Result:** Among 1503 samples, 108 were pathogenic. The most common isolate was *Klebsiella pneumoniae* [41.6%] followed by *Pseudomonas aeruginosa* [34.25%], *Escherichia coli* [21.29%], *Coagulase staphylococcus aureus* [1.85%] and *Acinetobacter spp* [0.92%] from sputum samples of patients suffering from upper or lower respiratory infection.

**Conclusion:** In our study *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* were the pathogens in all age groups and sexes.

**Keywords** – Bacteriological profile; Gram staining; Antimicrobial drug resistance; Culture; Respiratory tract infections; Sputum culture.

## 1. Introduction

Upper and lower respiratory tract infections are a significant cause of morbidity and mortality, especially in patients who are elderly, have prior lung disease, or have immune system suppression [1].

Sputum is the sample that is most frequently used to diagnose lower respiratory tract infections since it is simple to obtain and the method is non-invasive [2]. To find any potential respiratory infections, sputum samples are cultured and subjected to Gram stains [3].

Sputum culture is crucial for the practical and economical diagnosis of lower respiratory tract infections. The trustworthiness of the sample, however, is compromised by the possibility of oral flora and saliva contamination during sample collection. Inappropriately processing tainted samples that are useless for patient treatment also saves the wasteful use of resources and time [6]. The specificity of the sputum culture in the diagnosis of respiratory infection may rise if microscopy is used more frequently before culture.

Insufficient information about the etiology of lower respiratory infections is provided by the culture and gram stain due to poor correlation, which causes delays and indecision in patient management [7]. If the contamination by oropharyngeal flora has not been ruled out, the pathogen identified by isolation from sputum culture cannot be assumed to be the culprit that caused the respiratory infection. By inspecting the

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material under a microscope and counting the relative number of squamous epithelial cells in the sample, one can determine whether such contamination is present. Contaminated samples don't offer accurate diagnostic data for the patients ongoing care. Without first checking for potential contamination, interpreting sputum cultures might result in confusion and incorrect treatment decisions for patients with respiratory infections [8].

Before inoculating any sputum samples into the culture media, a routine gram stain is required to determine whether the sample is purulent. Sputum samples are examined under a microscope to prevent incorrect administration of culture in the treatment of patients with lower respiratory tract infections [9].

Infections of the lower respiratory tract (LRTIs) are widespread in the general population, although they are more likely to affect the elderly, people with chronic illnesses, and people whose immune systems are weak. Managing patients with LRTIs is a difficult task for a doctor because LRTIs are more common than ischemic heart disease, cancer, malaria, or human immunodeficiency virus infection [10].

The primary guidelines for treating LRTIs have been developed by various groups, including the British Thoracic Society (BTS) and the Infectious Disease Society of America (IDSA) [11, 12]. In order to treat LRTIs, it is crucial to identify the infectious organisms that cause them [12].

Infection can occur because of infectious agents including bacteria, virus, fungus, and protozoa. These agents encompass a wide range of organism such as [13]. *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Hemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus species*, *Histoplasma capsulatum*, *Candida albicans*. Most common organism are *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*.

The proper management of respiratory tract infections has been threatened by the changing antibiotic sensitivity pattern that is quickly growing as a result of over-the-counter sales and rising antibiotic overuse. The Centres for Disease Control (CDC) noted the significant financial impact that antibiotic-resistant illnesses bring. Infections that are resistant to first- and second-line therapies cost more to treat and require a longer hospital stay. According to estimates of up to 20 billion US dollars [14], antibiotic resistant illnesses cost the US economy.

Antibiotics received prior to producing the specimen, which happens in around 25% of cases [15], reduce the production of the sputum cultures even further. According to the American Thoracic Society's guidelines for infectious diseases, only adult hospitalised patients who can provide a high-quality pretreatment specimen and who can meet the quality performance measures for sputum collection, transport, and sample processing should be sampled [16].

Education of healthcare professionals and patient comprehension are necessary for a good sputum sample [17]. The culture of good quality sputum samples acquired from patients who underwent antibiotic treatment is probably not cost-effective [18]. Bacteria might develop resistance to one or more antibiotics during the treatment of bacterial illnesses [19]. Drug inactivation or modification, alteration of the target site, alteration of the metabolic route, and decreased drug accumulation are some of the ways by which organisms demonstrate antibiotic resistance [20]. From one country to another, as well as from one hospital to another, there are differences in the patterns of infection-causing bacteria and antibiotic resistance [21]. There have been reports of cross resistance and multiple resistance patterns worldwide [22].

The purpose of this study is to identify the causing bacteria from diverse sputum samples and investigate their susceptibility to various antibiotics.

## 2. Material and Method

**Study design** – Cross sectional study

**Study area** - This research was conducted at the microbiology department of Era's Lucknow Medical College and Hospital in Lucknow, Uttar Pradesh.

**Study population** – Patients attending the Era Medical College and Hospital's OPD and IPD in Lucknow.

**Study population and sampling method** - A total of 108 samples were collected from Era's Lucknow Medical College and Hospital, Lucknow, for analysis at the Department of Medical Microbiology laboratory after 108 clinically diagnosed patients were included in the study using a standard sampling technique.

**Inclusion criteria** – The patients who were thought to have a bacterial illness of the respiratory tract had their sputum collected from them all. The samples included were those that were obtained prior to the start of antibiotic medication.

**Exclusion criteria** - Sputum samples contaminated with saliva was excluded. Inadequate amount of sample.

### Study procedure

**Collection of sample:** Make the collection in a 100 ml or less capacity, widemouthed, screw-capped plastic container for disposal. Deliver the sample to the lab as soon as you can, preferably within two hours, as infections, especially sensitive bacteria, may go extinct with a longer delay [23].



Figure no 1: Sample container

**Microscopic examination:** The presence of epithelial cells, neutrophils, and polymorphs in pus cells.

**Sample Processing:** The direct gram stain and typical loop procedure were used to the sputum samples. Sputum was cultured on Blood Agar, MacConkey Agar, and Nutrient Agar. Overnight, plates were incubated at 37 °C.

Gram stained smear results were interpreted using microscopic observations of microorganisms, pus cells, and epithelial cells. The Bartlett grading system was used to evaluate the quality of expectorated sputum samples. The average number of epithelial and pus cells were counted in 20 to 30 low power fields (LPF), which is how the overall score was determined. The pus cells and epithelial cells were seen under a microscope.

A sputum sample that has been contaminated by saliva is considered to be unacceptable if the final score is less than or equal to zero. Sputum sample was regarded to be acceptable if the final score was 1 or higher [24].

Table no 1: Bartlett’s Criteria

Number of neutrophils / 10X LPF	GRADE
< 10	0
10 – 25	+1
>25	+2
Presence of mucus	+1
Number of Epithelial Cells / 10 X LPF	
10 – 25	-1
>25	-2
TOTAL SCORE	

Each bacterial isolate was generated according to the Clinical Laboratory Standards Institute's published instructions for susceptibility testing protocols.

### 3. Results

#### 3.1 Identification of bacteria

The typical colony morphology, pigment production, sugar fermentation (lactose fermenter and non-lactose fermenter), Gram staining, oxidase test, catalase test, oxidative fermentation test (for *Pseudomonas aeruginosa*), and biochemical tests were all used to identify the organisms.

#### 3.2 Isolation

In the bacteriological laboratory, all clinical specimens were received and cultured on Nutrient Agar, Blood Agar, and MacConkey Agar. The plates were incubated at 37 °C for 14 to 28 hours.

#### 3.3 Colony Characteristics

Colony of *klebsiella* on MacConkey agar plates: Due to the lactose fermentation, colonies were large, mucoid, dome shaped and sticky pink in color.

Colony of *Klebsiella* on blood agar colonies were non-hemolytic, show gamma hemolysis colonies of *Pseudomonas aeruginosa* characteristics on blood agar colonies are grey and moist and it produce beta hemolytic.

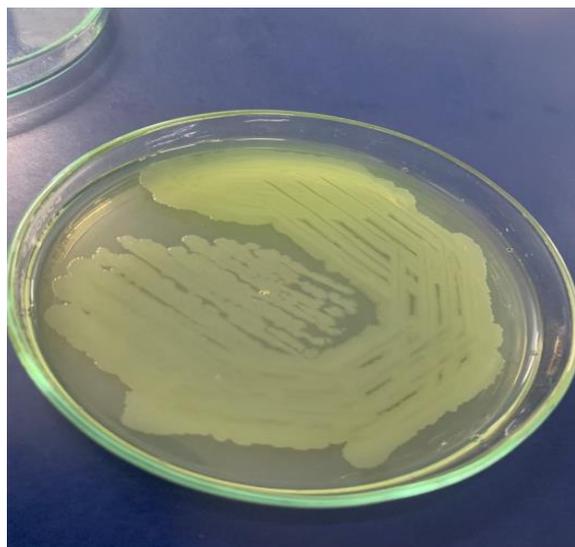
Colony of *Pseudomonas aeruginosa* characteristics on MacConkey agar, It produce pale non-lactose fermenting colonies.

Colony of *Pseudomonas aeruginosa* characteristics on nutrient agar:-Colonies showing greenish coloration due to production of Pyoverdine pigment and it produces large, opaque, irregular colonies with metallic sheen and distinctively fruity odour.

Colony of *Escherichia coli* characteristics on MacConkey agar:-It produces moist, flat and pink colonies due to lactose fermentation.



Figure 2 : Bacterial colony of *Klebsiella pneumoniae*



**Figure 3 :** Bacterial colony of *Pseudomonas aeruginosa*



**Figure 4:** Bacterial colony of *Escherichia. coli*

Using Gram stain, biochemical testing, and an antibiotic sensitivity test, identification was completed.

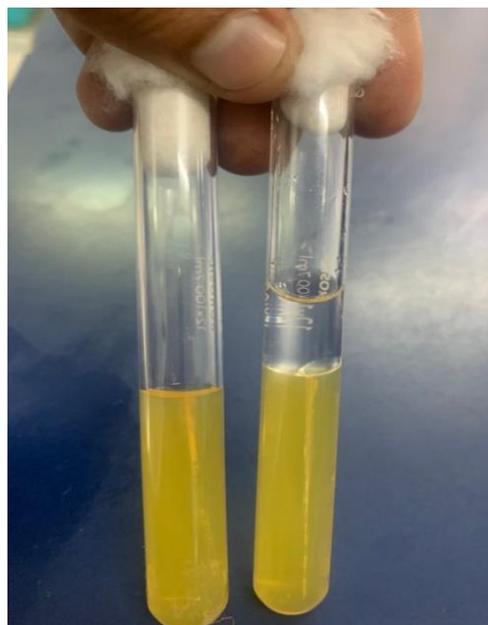
**Gram Staining** - Mackie and McCartney; practical medical microbiology 14th edition - 2021): 45:796 Gram staining is one of the special and differential staining of microbiology, discovered by Danish Bacteriologist Hans Christian Gram (1882) who identified by causing pneumonia.

**Biochemical Tests** - Various tests were performed, including MR, Indole, Urease, Citrate, and Triple Sugar Iron (TSI). Low levels of H<sub>2</sub>S were found by the TSI test. If sugar was fermented and gas was created after a heavy inoculum was streaked over the slope's surface and pierced into the butt during a 24-hour aerobic incubation at 37 °C, the reaction was positive. *Klebsiella pneumoniae*, (A/A). However, no H<sub>2</sub>S was created.

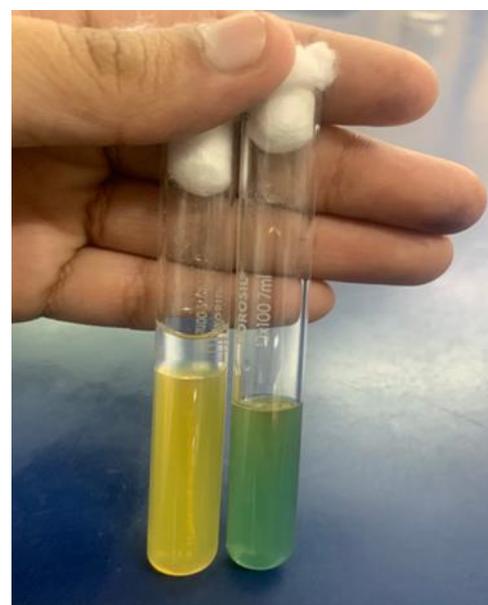
**Oxidative fermentation (OF) test:** Hugh and leifson OF test differentiates between fermenters and non-fermenters (that utilize sugars oxidatively).

Result: Oxidative for *pseudomonas* (figure no 5)

Fermentative for Enterobacteriaceae (figure no 6)



**Figure 5 :** OF Test for *Pseudomonas*



**Figure 6 :** OF Test for *Klebsiella pneumoniae Aeruginosa*

In our study involved the analysis of 1503 sputum samples, out of which 108 samples showed positive results indicating bacterial growth, while the remaining samples demonstrated no bacterial growth or normal flora. The quality of sputum was accessed using the Bartlett's methods.

Out of 108 samples 65(60.18%) were IPD patients and 43(39.81%) were OPD patients. Bacterial infection was more in males 71(65.74%) than females 37(34.25%). The most common age group 60 - 79 years[table no 3]. Out of 108 samples, *Klebsiella pneumoniae* (45) was the most common isolated organism followed by *Pseudomonas spp* (37), *Escherichia. coli* (23), *Coagulase negative staphylococcus* (2), *Acinetobacter spp* (1) [graph no. 2 ]

In *Klebsiella pneumoniae* the high sensitivity were seen in colistin (100%), tigecycline (100%), imipenem (91.11%), meropenem (92%), doripenem (92%) [graph no. 3].

In *Pseudomonas* the high sensitivity were seen in colistin (100%), imipenem (97.29%), meropenem (94.59%), piperacillin/ tazobactam (95%), doripenem (94%) [graph no. 4].

In *Escherichia coli* the high sensitivity were seen in colistin (100%), tigecycline (100%), imipenem (95.5%), amikacin (95.3%), meropenem (91.30%) [graph no. 5].

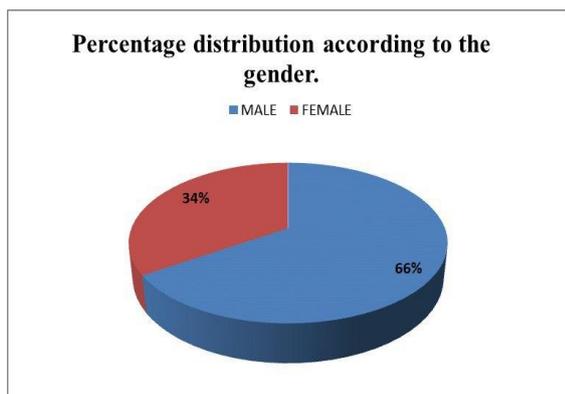
In CONS the 100% sensitivity in gentamicin, linezolid, vancomycin, amikacin and teicoplanin [graph no. 6].

**Table no 2 :-** Distribution of patients in IPD and OPD

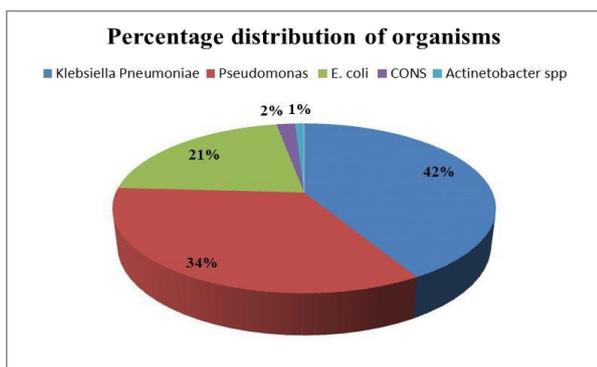
S. No	Wards	NUMBER	PERCENTAGE
1.	IPD	65	60.18%
2.	OPD	43	39.81%

**Table 3 :-** Showing distribution according to age-wise

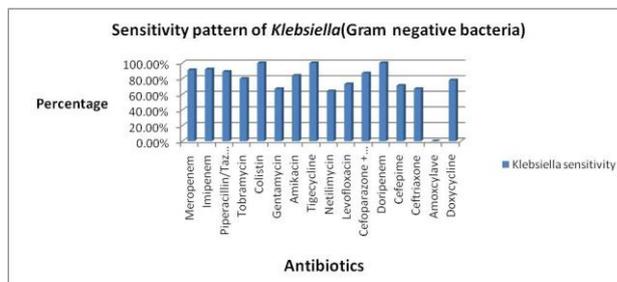
S.NO	AGE(YEAR)	NUMBER	PERCENTAGE
1	0 – 19	6	5.5%
2	20-39	19	17.59%
3	40-59	27	25%
4	60-79	51	47.2%
5	80-99	5	4.6%



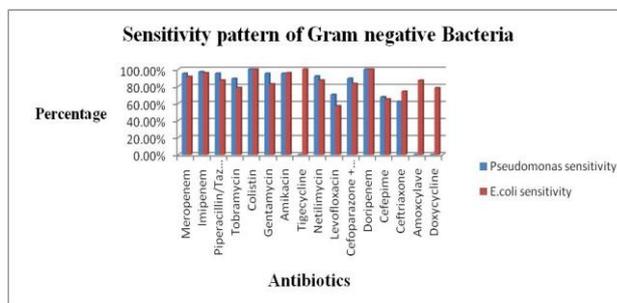
**Graph no 1:-** Percentage distribution of gender



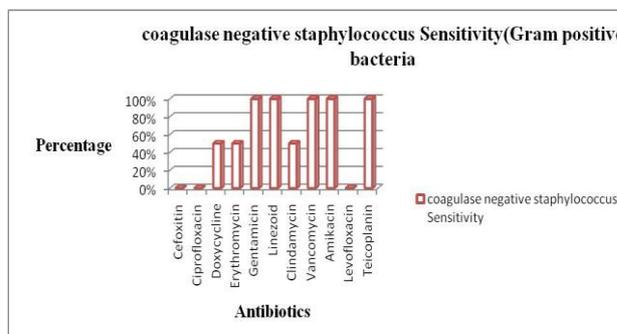
**Graph no 2:-** Percentage distribution of organisms



**Graph 3:-** Shows sensitivity pattern of *Klebsiella pneumoniae*



**Graph 4:-** Shows sensitivity pattern of gram negative bacteria



**Graph 5:-** Shows sensitivity Pattern of *Coagulase negative Staphylococcus aureus*

**4. Discussion**

The objective of the current study was to assess the most prevalent microorganism and examine the sputum samples antibiotic susceptibility patterns. There were 108 instances included in the study in all. For the Department of Microbiology at Era's Lucknow Medical College & Hospital, patient samples were collected through OPDs and IPDs.

The other objectives were:-

1. To isolate and identify the common organisms from sputum samples.
  2. To determine the antimicrobial susceptibility pattern of the various isolated organisms.
- In the present study males (65.74%) were more affected than females. The finding is in accordance with Akansha et al[25] (73.4%) and in contrast with

Dhivya G *et al*[24] (59%) males were affected than females. Males were affected more frequently than females due to the fact that men engage in outside activities and professions more frequently than women. The danger of infection is increased by these pursuits and careers.

In our study the highest percent in age between 60 – 79 years and is comparable to Haroon [27] study the highest percent in age between 55 – 65 years and similarly study done by Haider *et al* [25] the highest percentage between 50 – 60 years. Weaker immune system was the root of the problem.

Regarding the organism isolated from sputum culture in our study, commonest isolate obtained in the study were *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*. Which is similar to the Raghubanshi *et al*[28] *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter spp*, *Staphylococci*. In Nithya Chinnusamy *et al*[29] study *Klebsiella pneumoniae*, *Pseudomonas spp*, *Staphylococcus aureus*. The hospital's microbial profile, which in turn depends on the kind of procedure being performed there and the patient profile—which includes a higher number of OPD and IPD patients as well as post-surgical patients—was the cause of the most frequent isolates in hospital acquired infections (HAI) patients. Additionally, in surgical patients.

In our study gram positive antimicrobial susceptibility pattern observe in a hospital for *Klebsiella pneumoniae* was in case of sensitivity to 100% in colistin, doripenem followed by meropenem (91.11%), imipenem (92%), piperacillin – tazobactam (89%), amikacin (84%). Whereas Raghubanshi *et al.*, [28] showed the high sensitivity to Imipenem (86.9%), amikacin (83.63%) and gentamicin (71.51%) and high resistance to ampicillin (97.53%), amoxicillin – clavulanic acid (97.53%), ciprofloxacin (71.6%), cotrimoxazole (58.14%), ceftriaxone (58.92%), cefotaxime (52.89%) were moderately effective%. The study conducted by Debnath S *et al.*, [30] *Klebsiella* was sensitive to amikacin, imipenem, gentamycin, levofloxacin, ciprofloxacin, piperacillin and this association was statistically significant.

In our study *Pseudomonas*, second common organism, also an important causative agent for nosocomial infection, was found to be sensitive to doripenem (94%), imipenem (97.59%), meropenem (94.59%), tobramycin (89.18%), amikacin (94.59%), gentamicin (94.59%). Followed by cefepime (67.56%), ceftriaxon (62.16%). The other study of Debnath[30] study *Pseudomonas aeruginosa* was mainly sensitive to piperacillin, gentamicin and amikacin.

In another study done in Pakistan on resistance[31] patterns of *Pseudomonas species* isolated from burn patients demonstrated resistance towards gentamicin (92.85%) and ceftazidime (71%). In our present study resistance were amoxicillin - clavulanic acid (100%), doxycycline (100%), ceftriaxone (37.9%), levofloxacin (29.72%).

In our study *Escherichia coli* is sensitive to meropenem (100%), imipenem (100%), colistin (100%), tigecycline (100%) and doripenem (100%) and resistant to levofloxacin (43.47%), cefepime (34.78%), ceftriaxone (30.43%). In similar study of Haider *et al.*, [25] amikacin and imipenem both showed great sensitivity, while ACM, CTX, and CRO showed significant resistance.

In the study conducted by Ashish[32] the sensitivity to ertapenem (72.91%), imipenem (85.41%), tigecycline (70.83%), amikacin (62.4%). And a another five year retrospective study done in Cambodia[33] on *Escherichia coli* showed high resistance to ampicillin (96%) and cotrimoxazole (86%).

In our study shows that the gram positive bacteria were highly sensitive for vancomycin (100%), gentamycin (100%), linezolid (100%). And a study done by Raghubanshi *et al.*, [28] Vancomycin was observed to be 100% effective but this is only used for gram positive bacteria. Similarly in a study done by Oberoi *et al.*, [34]. Vancomycin seemed to be 92.7% effective.

In the study of Nasim O[35] the *Acinetobacter* species were extremely resistant to the antibiotics amikacin and ciprofloxacin, somewhat resistant to the antibiotics piperacillin/tazobactam, and very susceptible to the antibiotic colistin. Piperacillin, gentamycin, amikacin, netilmicin, colistin, levofloxacin, ciprofloxacin, and doxycycline were all highly ineffective against the *Acinetobacter* species in our study.

## Conclusion

- In the present study the bacteriological laboratory received 1503 sputum samples from patients who were thought to have bacterial illnesses and processed them for culture. 108 (100%) of the sputum samples tested positive for bacterial growth. .
- From sputum samples of individuals with upper or lower respiratory infections, *Klebsiella pneumoniae* was the most frequently isolated organism (41.6%), followed by *Pseudomonas aeruginosa* and *Escherichia coli*.
- Sputum samples were isolated frequently from patients who were male (65.74%) and from those who were between the ages of 60 and 79 (47.2%).
- The most sensitive drugs were imipenem, meropenem, piperacillin – tazobactam combination and gentamicin, colistin and doripenem.
- The Gram positive bacteria *Coagulase Negative Staphylococcus* most sensitive drugs were vancomycin, linezolid, teicoplanin, amikacin. In *Acinetobacter* most sensitive drug was colistin.
- To reduce the inappropriate use of antibiotics, proper diagnosis, identification of the causing

agents, and knowledge of their antimicrobial sensitivity pattern were crucial. The results of this investigation and other comparable studies are sufficient to describe the majority of the antimicrobials.

- It was useful for choosing an empirical course of treatment while waiting for the results of the patient's antimicrobial susceptibility tests.
- A study also emphasises the need for every hospital establishment to have a periodic antibiogram.

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