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Profile and antimicrobial susceptibility pattern of bacteria isolated from sputum samples in a tertiary care hospital

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Abstract
Background*: Wide range of pathogens are responsible for causing lower respiratory tract infection and it is a leading cause of human disease-causing high morbidity and mortality worldwide. Excessive and irrational use of antibiotics leads in developing of resistance in gram positive and gram-negative organisms, thus resulting in the emergence of pathogens which are difficult to treat.*
Objective: Aim of the study was to isolate bacteria causing lower respiratory tract infection and their antimicrobial susceptibility patterns from sputum sample in a tertiary care hospital
Materials and Methods*: All sputum samples submitted for culture from 01 Jan 2024 to 31 Dec 2024 were collected from patients attending People's Hospital, Bhopal using the standard sampling technique. Anti-microbial susceptibility testing of microbial isolates was performed according to the guidelines issued by the National Committee for Clinical Laboratory Standards.*
Results*: The most commonly isolated pathogen was Pseudomonas aeruginosa (n=35) followed by Klebsiella pneumoniae (n=28), Escherichia coli (n=25), Staphylococcus aureus (n=25), and Acinetobacterbaumanii (n=15) Pseudomonas aeruginosa was susceptible to most of the drugs and showed resistance towards gentamicin (48.6%) and ciprofloxacin (54.3%). It showed (100%) susceptibility to aztreonam and meropenem. Escherichia coli was 100% resistant towards ampicillin, co-trimoxazole, cefixime, and doxycycline. Least resistance was seen to piperacillin/ tazobactam (92%), cefepime (84%), imipenem (92%) and gentamicin (88%).*
Conclusion:*This study revealed that Pseudomonas aeruginosa and Klebsiella pneumonia were the commonest isolated organism from the sputum samples of patients suffering from lower respiratory tract infections. There were a significantly high proportion of patients who were affected by drug resistant strains. Before starting empirical antibiotics on patients with moderate to severe lower respiratory tract infections, it is always advisable to obtain sputum for culture and antibiotic sensitivity test.*
Key Word*: Sputum, Pseudomonas aeruginosa, lower respiratory tract infection*

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I. Introduction

Upper and lower respiratory tract infections are a cause of substantial morbidity and mortality, especially in elderly patients, those having prior lung disease, or are immunocompromised.[1]

Lower respiratory tract infections are considered 3rd leading cause of death worldwide, next only to ischemic heart disease and cerebrovascular diseases. These diseases, many a times, have non-specific presentations which leads to their misdiagnosis and mistreatment. This is more of a concern in the developing countries. Factors like geographical location, climate, socioeconomic status, age and associated risk factors contribute to the spectrum of lower respiratory tract infections.[2]

Most of the lower respiratory tract pathogens are diagnosed by utilizing the sputum sample as it can be obtained non-invasively and is relatively simple to obtain if the patients are given adequate instructions. Nevertheless, the role of sputum culture has been debatable since the quality of sample may be compromised and repeated sampling may be necessary. Before inoculating any sputum samples into the culture media, a routine gram stain is done to determine whether the sample is purulent and the Bartlett's Criteria is used to screen out unsatisfactory sputum samples. [3]

Infection can occur because of infectious agents including bacteria, virus, fungus, and protozoa. These agents encompass a wide range of organism such as Streptococcus pneumonia, Staphylococcus aureus, Hemophilus influenza, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Proteus species, Histoplasma capsulatum and Candida albicans. [4] This wide range of etiological agents are a challenge to the clinicians to establish a specific diagnosis in order to start appropriate antimicrobial therapy.[5]

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. There have been reports of cross resistance and multiple resistance patterns worldwide [6].

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. Infections that are resistant to first- and second-line therapies cost more to treat and require a longer hospital stay.

The purpose of this study was to identify the bacteria from sputum samples of cases with lower respiratory tract infection and investigate their susceptibility to various antibiotics.

II. Material And Methods

Study design – Cross sectional study

Study location- This study was conducted in the Department of Microbiology People's College of Medical Sciences and Research Center, People's University, Bhopal

Study Duration:01 Jan 2024 to 31 Dec 2024.

Study Size– 352 sputum sample received during study period

Study population and sampling method – All sputum samples submitted for culture during the study period were collected from patients attending People's Hospital, Bhopal using the standard sampling technique

Inclusion criteria –All non-duplicate sputum samples obtained prior to the initiation of antibiotic therapy.

Exclusion criteria - Samples that were mostly contaminated with saliva and inadequate amount of sample were excluded

Study procedure

Collection of samples: Samples were collected in sterile wide-mouthed, screw-capped plastic container and transported to the laboratory as soon as possible, preferably within two hours[7].

Microscopic examination: Direct microscopic examination by Gram staining was performed and the presence of epithelial cells, neutrophils, and polymorphs was noted. 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), and the overall score was determined. A score of >1 indicates active inflammation while a score of 0 or less indicates a lack of inflammation or contamination with saliva.

Sample Processing:

Sputum was cultured on Blood Agar and MacConkey Agar and plates were incubated at 37 °C for 18 – 24 hours.

The typical colony morphology, pigment production, Gram staining, oxidase test, catalase test, sugar fermentation, oxidative fermentation test and other biochemical tests were all used to identify the organisms. [7]

Antimicrobial susceptibility testing was performed by disc diffusion method as per CLSI 2024 guidelines. [8]

III. Result

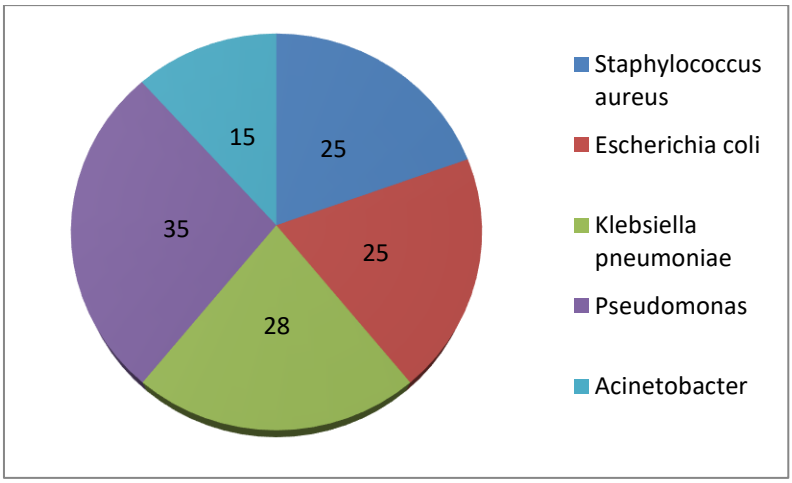
A total of 352 sputum samples were processed during the study period and 128 of them showed the growth of pathogenic bacteria. Among them, 35 (27.3%) were Pseudomonas aeruginosa, 28 (21.9%) were Klebsiella pneumoniae and 25 (19.5%) were Escherichia coli and Staphylococcus aureus each, and 15 (11.7%) were Acinetobacterbaumanii as shown in table no.1 and figure no. 1

Among the 128 positive samples, 82 (64%) were from male patients and 46 (36%) were from female patients.

Table No. 1: Number of isolates from sputum samples

Isolates	Number
Pseudomonas aeruginosa	35
Klebsiella pneumoniae	28
Escherichia coli	25
Staphylococcus aureus	25
Acinetobacterbaumanii	15
Total	128

Figure No. 1: Number of isolates from sputum samples

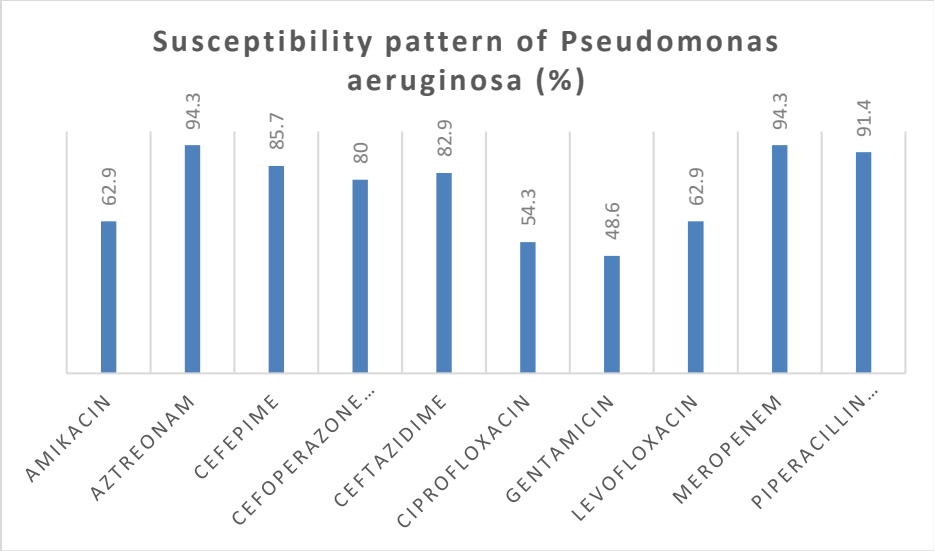


In this study, it was observed that among Pseudomonas aeruginosa isolated, 94.3% isolates were susceptible to meropenem and aztreonam and 91.4% to Piperacillin/ tazobactam. 48.6% were resistant to gentamicin and 54.3% ciprofloxacin as shown in table no.2 and figure no.2.

Table no. 2. Susceptibility pattern of Pseudomonas aeruginosa in percentage

Antibiotics	Susceptibility % (n=35)
Amikacin	62.9
Aztreonam	94.3
Cefepime	85.7
Cefoperazonesulbactam	80
Ceftazidime	82.9
Ciprofloxacin	54.3
Gentamicin	48.6
Levofloxacin	62.9
Meropenem	94.3
Piperacillintazobactam	91.4

Figure No. 2: Susceptibility pattern of Pseudomonas aeruginosa in percentage



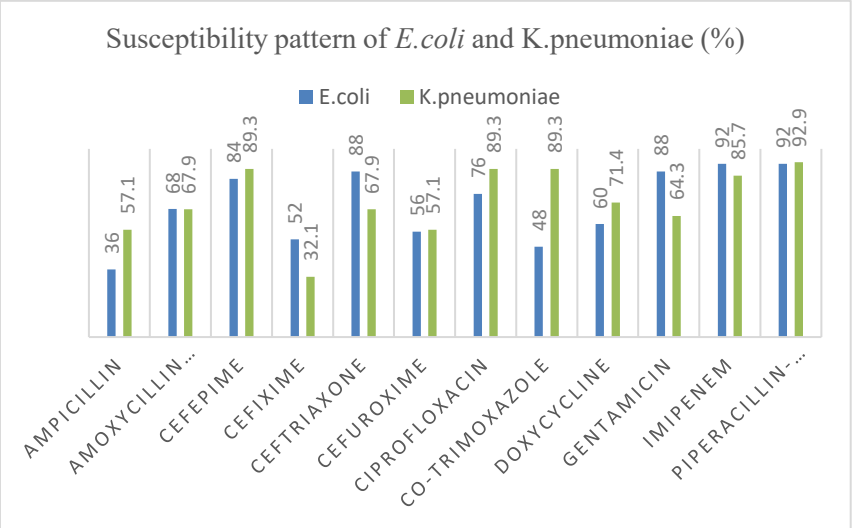
Among the Klebsiella pneumoniae isolated, 89.3% were susceptible to cefepime, and ciprofloxacin while 25.6% were resistant to cotrimoxazole and 32.1% to cefixime as shown in table no.3. Escherichia coli isolated were

susceptible to piperacillin/ tazobactam and imipenem (92% each) while resistant 36% were resistant to ampicillin as shown in table no 3 and figure no.3.

Table no.3. Susceptibility pattern of E. coli and K. pneumoniae in percentage

Antibiotic	K. pneumoniae Susceptibility % (n=28)	E. coli Susceptibility % (n=25)
Ampicillin	57.1	36
AmoxycillinClavulanic acid	67.9	68
Cefepime	89.3	84
Cefixime	32.1	52
Ceftriaxone	67.9	88
Cefuroxime	57.1	56
Ciprofloxacin	89.3	76
Co-trimoxazole	28.6	48
Doxycycline	71.4	60
Gentamicin	64.3	88
Imipenem	85.7	92
Piperacillin-tazobactam	92.9	92

Figure No. 3: Susceptibility pattern of E. coli and K. pneumoniae in percentage

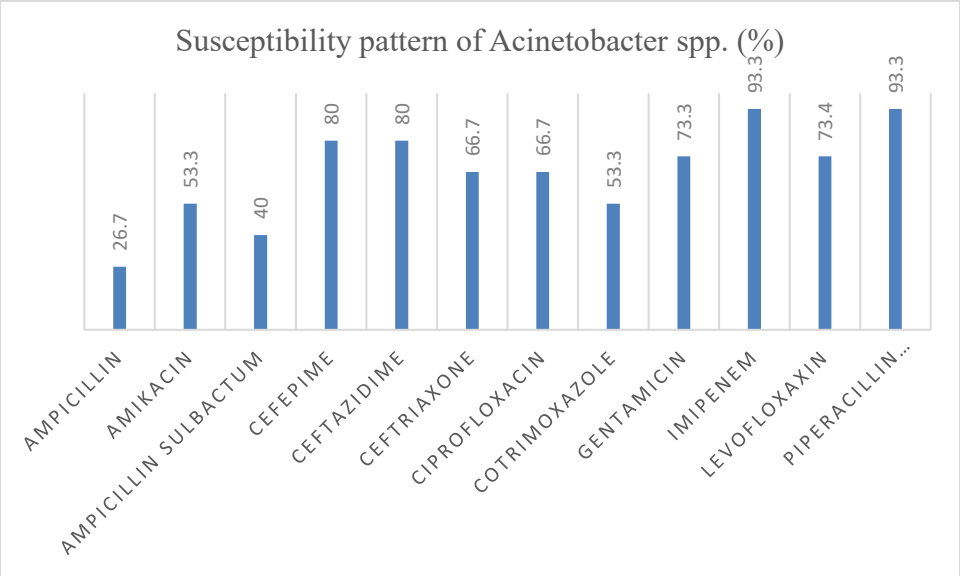


In this study it was observed that among the Acinetobacterbaumanii isolated, 93.3% were susceptible to Piperacillin/ tazobactam and imipenem while 26.7% were resistant to ampicillin as shown in table no 4 and figure no.4.

Table No. 4. Susceptibility pattern of Acinetobacterspp in percentage

Antibiotics	Susceptibility % (n=15)
Ampicillin	26.7
Amikacin	53.3
Ampicillin sulbactam	40
Cefepime	80
Ceftazidime	80
Ceftriaxone	66.7
Ciprofloxacin	66.7
Cotrimoxazole	53.3
Gentamycin	73.3
Imipenem	93.3
Levofloxacin	73.4
Piperacillintazobactam	93.3

Figure No. 4. Susceptibility pattern of Acinetobacter baumanii in percentage

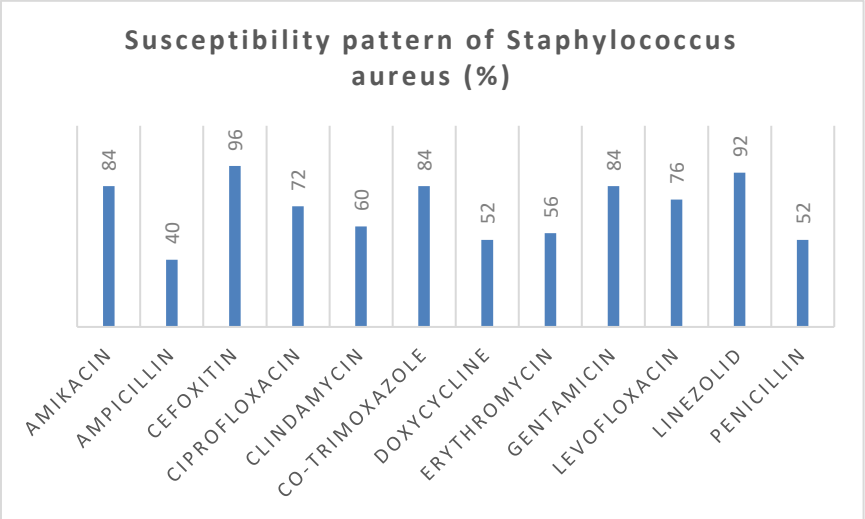


Staphylococcus aureus isolated were found to susceptible to cefoxitin (96%) and linezolid (92%) while resistant to penicillin and doxycycline (52% each) as shown in table no 4 and figure no.4.

Table no. 5. Susceptibility pattern of Staphylococcus aureus in percentage

Antibiotics	Sensitivity % (n=25)
Amikacin	84
Ampicillin	40
Cefoxitin	96
Ciprofloxacin	72
Clindamycin	60
Co-trimoxazole	84
Doxycycline	52
Erythromycin	56
Gentamicin	84
Levofloxacin	76
Linezolid	92
Penicillin	52

Figure no. 5. Susceptibility pattern of Staphylococcus aureus in percentage



IV. Discussion

The objective of the current study was to assess the most prevalent microorganism and their antibiotic susceptibility patterns. There were 352 samples that were included in the study that were submitted to the Department of Microbiology at People's College of Medical Sciences and Research Center, Bhopal.

In the present study, 36.7% culture positivity was observed, while it was 43.3 % in study by Singh S *et al*[2] and 48% in Kumar M *et al*. [9]In other studies from Ethiopia, GebreAb et al [2] has reported positivity of 35% [10] and Nurahmed N *et al* has reported 32% [11]. A study from Nepal by Khan S *et al* has reported 49.3% [12] culture positivity. The positivity percentage was similar in all the studies.

LRTIs were common in male patients as compared to female patients (71.1% v/s 28.9%) in present study which is comparable with the other Indian studies like, Singh S et al [2], Kumar M et al [9] and Dhivya G et al. [13]

In our study, out of the 128 positive culture samples, Pseudomonas aeruginosa (n=35, 27.3%) was the most common pathogen isolate followed by Klebsiella pneumonia (n=28, 21.8%), Escherichia coli (n=25, 19.5%), Staphylococcus aureus (n=25, 19.5%) and Acinetobacterspp (n=15, 11.7%). This was similar to study done by Asati et al and Edirishinghe LU et al. [14,15]

In contrast, in another study of sputum culture isolates yielded Klebsiella pneumonia in majority (30.4%) [16] similar to reports from Nigeria (34.29%) [17], Tanzania (29.9%) [18], and Ethiopia (25.4–39.5%). [10]

In another study, Escherichia coli was most common (43.3%) followed by Staphylococcus aureus (21.1%), Klebsiella pneumoniae (8.9%), Pseudomonas aeruginosa (6.7%) and Acinetobacterspp (5.7%).[19]

In this study, it was observed that among Pseudomonas aeruginosa isolated, 94.3% isolates were susceptible to meropenem and aztreonam and 91.4% to piperacillin/ tazobactam. While in study by Shilpa et al. it wasfound to be susceptible to imipenem, amikacin, ciprofloxacin, gentamicin and [20] showed susceptibility to gentamicin, meropenem and ciprofloxacin.

Among the Klebsiella pneumoniae isolated, 89.3% were susceptible to cefepime, and ciprofloxacin, while in other studies it has been found susceptible to imipenem (86.9%), amikacin (83.63%) and gentamicin (71.51%) [21]. A study done byEdirisinghe, et al[15]. showed 66.66%, 62.50% and 56.66% sensitivity towards Amikacin, Gentamicin, and Imipenem respectively and Usman et al found susceptibility to ciprofloxacin, ceftriaxone and meropenem.[17]

Escherichia coli isolated were susceptible to piperacillin/ tazobactam and imipenem (92% each) while resistant 36% were resistant to ampicillin while in study by Esha Gupta et al., Escherichia coli was susceptible to imipenem (95.5%), amikacin (95.3%), meropenem (91.30%).[22]

In this study it was observed that among the Acinetobacterbaumanii isolated, 93.3% were susceptible to Piperacillin/ tazobactam and imipenem while 26.7% were resistant to ampicillin. Singh, J et al showed that 80% resistance to carbapenems.[2]Gebre et al found 89.5% susceptible to cefepime and 84.2% to ceftriaxone.[10]

Staphylococcus aureus isolated were found to susceptible to cefoxitin (96%) and linezolid (92%) while resistant to penicillin and doxycycline (52% each) while Singh J et al., reported 100% resistance to penicillin and 100% susceptibility vancomycin and linezolid.[2] Khan S et al reported, among the 28 isolates, 92.86% resistance to penicillin and 82.14% resistance to cotrimoxazole while 89.3% were susceptible to ciprofloxacin, [12]which was similar to study done by Kumar M et al. [9]. Gebre et al found 90.6% susceptibility to cefoxitin and 76.2% to gentamicin which was similar to the present study.[10]

So, there is wide range of variability in the type of isolates from sputum samples and the susceptibility patterns of bacteria isolated, from different geographical areas. Hence it is necessary to conduct more such studies to give relevance to local isolates and form antibiotic policies accordingly.

V. Conclusion

This study revealed that Pseudomonas aeruginosa and Klebsiella pneumonia were the commonest isolated organism from the sputum samples of patients suffering from lower respiratory tract infections. There were a significantly high proportion of patients who were affected by drug resistant strains. Before starting empirical antibiotics on patients with moderate to severe lower respiratory tract infections, it is always advisable to obtain sputum for culture and antibiotic sensitivity test.

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