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Research Article

Antibiogram Pattern of Bacterial Isolates from Sputum Sample

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Abstract

Antibiotics are frequently used for various infectious disease example; acute lower respiratory tractinfection. But, injudicious use of antibiotic resistance is an emerging problem. The objective of this study was taken up to analyze the antibiogram pattern of bacterial isolated from the sputum samples of patient in Manmohan Memorial Medical College and Teaching Hospital. Initially, 250 sputum specimens of patients were collected and opted for the isolation and identification of isolates which detected 56.097% *Klebsiella spp.* 17.073 %, *Pseudomonas aeruginosa* 4.878 %, *Burkholderia spp*. 9.756 % *Enterobacter spp.* 12.195% *Escherichia coli* respectively. ConductingAST following Kirby Bauer disc diffusion method (CLSI 2020), it was determined that *Klebsiellaspp.* (56.097 %) exhibited sensitive to Gentamycin and exhibited resistance to Cefixime. It was observed that 9 (39.130 %) *Klebsiella spp.* isolates exhibited multi-drug resistance character. Likewise, out of 23 isolated *Klebsiella spp.* only 5 (21.739 %) was found to be ESBL producers. The presence of multi drug-resistant strains and Extended Spectrum Beta-Lactamase (ESBL) producing *Klebsiella spp.* is of major concern worldwide. The improper and excessive utilization of antibiotics, encompassing unauthorized practices might be the reason in the rise to the development and proliferation of antimicrobial resistance (AMR) and multidrug resistance (MDR)in microorganisms. This study highlights the importance of implementing appropriate hygiene and dietary measures for human to prevent the transmission of diverse pathogenic microorganisms.

Keywords: *Klebsiella* spp., Multi-drug resistance, Antimicrobial resistance, ESBL.

Introduction

The type of mucus secreted by cells in the lower airways bronchial and bronchioles of respiratory tract is called sputum or phlegm. Sputum is different from saliva that rises up into the mouth. Deadcells, foreign particles inhaled into lungs, bacteria and white blood cells that protect the airways from infection may be present in the sputum. Diagnosis of lung cancer and other medical conditions affecting sputum production can be helped by the quantity, texture and color of sputum. (Lynne, 2023). Sputum can be of several different colors. The colors can help identify the type of infection or a chronic illness has become worse. Purulent type of sputum with thick, yellow or green character indicates infectious pneumonia, bronchiectasis and abscess. Similarly, mucoid typesputum with clear, grey or white character indicates chronic obstructive pulmonary disease and asthma. Blood in sputum indicate malignancy, pulmonary embolus, clotting disorders, infections and many more (Education, 2015).

Sputum is the most common specimen collected from patients with lower respiratory tract infection and is important for bacteriological identifications, which covers a wide range of diseases and are a major concern in clinical practices and

medical researches because of their contributionto mortality in elderly patients and with comorbidities (Zhou *et al.*, 2010). Infections of the lowerrespiratory 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 (Popova *et al.*, 2019). The identification of pathogens plays an important role in the treatment of infection. An accurate and rapid identification method can help guide the use of narrow-spectrum antibiotics to reduce economic burden, reduce bacterial symbiosis and avoid the emergence of resistant bacteria (Zhou *et al.*, 2010).

Any microorganisms found in sputum are the sign of lower respiratory tract infection. Mycobacterium tuberculosis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,Staphylococcus aureus, Streptococcus pneumoniae etc. are the possible pathogens found in sputum specimen. Several studies from around the world report that the potent pathogens of AGI are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus spp*., *Moraxella catarrhalis*, and *Streptococcus pyogenes*. Other Gram-negative bacteria in the gut such as *Salmonella choleraesuis*, *Citrobacter koseri*. Most of these bacteria are part of the normalflora in the human respiratory tract. Thus, it is clear that most of the time infection is initiated by normal flora and secondary infection by other invading bacteria (Regmi *et al*., 2020).

Sputum analysis and culture are the most common methods for the specific diagnosis of lower respiratory tract infections. A culture of sputum sample that is examined and properly spit up willidentify the causative agent in the most cases of bacterial pneumonia. Sputum produced as well assputum obtained from upper respiratory tract is susceptible to bacterial infection of the oropharynx.(Joyce, 1986). Managing patients with LRTIs is very difficult for clinicians since LRTIs provide ahigher global health threat than ischemic heart disease, cancer, malaria, or HIV infection (Raghubanshi & Karki, 2020).

The first stage of sputum analysis in a lab is smear microscopy. In environments where resourcesare scarce, it is a quick and affordable procedure. Gram-positive and gram-negative microorganisms are the two main kinds of bacteria that are distinguished by the Gram stain. The Gram stain, which is the initial staining method used in preliminary bacterial identification, aids in determining whether there are enough pathogens in the culture to provide a conclusivediagnosis. It is also essential since it allows for a more focused discussion of antibiotic therapy. Acid-fast bacilli (AFB) stain testing must be done when a doctor suspects that a patient may have TB (Popova *et al.*, 2019). The next stage in identifying the bacteria is to conduct biochemical testsof bacterial growth. Motility, McFarland standard, Triple Sugar Iron Agar, Citrate, catalase, and oxidase assays are typical biochemical tests used to determine bacterial growth. (Manandhar & Sharma, 2018). Lower respiratory tract infections (LRTIs) make up over 90% of all human illnesses that are recorded as respiratory tract infections (RTIs). Failure to distinguish TB from other LRTIs may result in poorer health outcomes and greater mortality (Regmi *et al.*, 2020).

An infection of lower respiratory tract is typical and causes a larger burden of disease globally. The developing antimicrobial resistance poses an even greater difficulty to practitioner (Raghubanshi & Karki, 2020). As there is the increment in the rate of AMR, it becomes a major threat to a developing country like Nepal. Therefore, this study highlights the issue of taking corrective measures to mitigate the consequences. The study also helps in spreading awareness tothe public and pertinent authorities about the implications of antimicrobial misuse, which can prevent cases of antibiotic resistance in patients to some extent. Examining the degree of resistancecan be regarded as a reliable gauge for assessing the presence of antibiotic resistance.

Materials and Methods

Sputum samples of patients were collected from Manmohan Memorial Medical College and Teaching Hospital. Samples were collected and opted for the isolation and identification of the isolates using different test procedure.

The research was conducted from May 2024 to August 2024 at Department of Microbiology, Trichandra Campus, Kathmandu. A laboratory-based descriptive cross-sectional study. The sampling technique used was purposive sampling, where samples were collected considering the purpose of study.

Sputum Culture Procedure

Sputum samples were placed on culture plates containing specific substances that promote bacterial or fungal growth. Then, the dish was covered with a lid and placed in an incubator at 37°C for bacteria and 30 °C for fungi. Bacterial or fungal growth in the sputum plate was checked. After a positive sputum culture result, microscopy, colony morphology, or biochemical tests for bacterial growth were done to identify specific types of bacteria or fungi.

Sputum Stain Test Procedure

A sputum sample was swabbed on a slide. The various stains were added to sample cells, bacteria,and fungi on slides and washed with water, alcohol, or acid solutions. Then, the slides were examined under a microscope.

Sputum Biochemistry Procedure

Bacteria was first inoculated onto a series of different media. An indicator was used to observe specific metabolic end products in the medium.

Sputum Cytology Procedure

The sputum samples were swabbed on slides and stained with different dyes according to the instructions. The stained slides were then examined under a microscope.

Testing Procedure for Nucleic Acid Amplification in Sputum

RNA or DNA was extracted from sputum samples according to the instructions of commercial kits as Qiagen QIAamp Mini kit and Qiagen RNeasy Mini. DNA or RNA was placed in PCR reaction tubes along with designed primers, Taq polymerase, deoxynucleoside triphosphates (dNTPs), and fluorescently labeled probes. The tube containing the RT-PCR reaction mixture was then placed in a real-time PCR machine to amplify the molecules at a specific temperature.

Procedures for Antimicrobial Susceptibility Testing in Sputum

In the MIC method, bacteria or fungi isolated from sputum samples were diluted in saline and applied to the MIC panel. In the dish diffusion method, different concentrations of selected antibiotics were added directly to the bacteria-spotted agar plate. Plates were incubated at 35°C for approximately 16–18 hours or more. The minimum concentration of antibiotics that inhibit microbial growth, or MIC panels, was read according to various manufacturers' guidelines. The results were then recorded.

Sputum Cytology Procedure

The sputum samples were swabbed on the slides and stained with different dyes. Then, it was examined to find abnormal cells in the sample.

Minimum Inhibitor Concentration

In the MIC method, bacteria or fungi isolated from sputum samples were diluted in saline and applied to the MIC panel. In the dish diffusion method, different concentrations of selected antibiotics are added directly to the bacteria-spotted agar plate. Plates are incubated at 35°C for approximately 16-18 hours or more. The minimum concentration of antibiotics that inhibits microbial growth, or MIC panels, are read according to various manufacturers' guidelines. The results are then reported (Shen *et al.*, 2023).

Result and Discussions

Distribution of Growth Pattern of Bacteria

250 sputum samples were cultured. Out of them, 20% were significant growth and 80% of them were non-significant growth from the patients visiting Manmohan Hospital's Molecular Laboratory (Fig. 1).

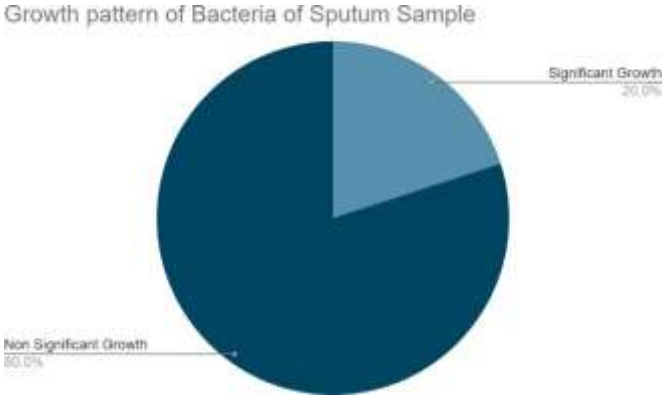

Fig. 1: Growth pattern of Bacteria of Sputum Sample

The study aimed to investigate the antibiogram pattern of bacterial isolates from sputum specimens were collected. One study was conducted, which showed out of 198 patients screened, 48 were sputum smear-positive and 83 were negative leaving sixty-seven excluded for various reasons. Similar study was carried out by (KC *et al.*, 2018) at Sukraraj Tropical and Infectious Disease Hospital (STIDH) from August 2017 to March 2018, 24 positive cases and 76 negative cases were found out of 100 sputum samples. On our finding, the relatively low percentage (20%) of significant growth on sputum specimens was seen, which is comparatively lower than other research findings.

Age wise and of patients visiting Manmohan Hospital

Total 250 samples were proceeded out of which 50 showed significant growth. Among them, maximum significant growth was seen within age group 71-80 followed by age group 81-90 and 51-60. The age group of 91-100 shows no growth at all (Table 1).

Table 1: Bacterial Sample according to age group

Age Group in year	Positive Growth	
	Number	Percentage
11-20	2	4
21-30	3	6
31-40	6	12
41-50	5	10
51-60	8	16
61-70	3	6
71-80	15	30
81-90	8	16
91-100	0	0
Total	50	

The age distribution reveals that the highest number of patients fall within the 71-80 age group, where 15 people showed positive above 70 and 8 people above 81-90 showed significant growth. It may be because people of that age group are sensitive to Bacterial infection. The study from the far western region shows the similar result where out of 280 smears from suspected patients, only 30 showed smear positivity (10.7%). In a published report of Dhakal *et al.* (2018), among the total identified positive cases of respiratory infections (30/280), the highestrate was observed in the age group of 56-65 years. Another study which was conducted in Manmohan Memorial Hospital published in the journal "BMC Pulmonary Medicine" (Shrestha *et al*, 2022), where out of 300 samples the maximum positives i.e.40 % were seen from the age group over 45 years old. It also states that male tend to have slightly higher infection compared to women.

Gender Wise Distribution Of Bacterial Growth

Out of 250 samples, the 50 samples showed significant cultural growth, out of which 54% were ofmale patients and the rest 46% were of female patients (Table 2).

During AFB staining procedure, 7 were found AFB positive among which 4 were of male and rest 3 were of female. Further

procedure was excluded and we refer AFB positive samples to TB hospital. There was a study of patients by Pariyar *et al.*, (2023), visiting the District Public Health Office, where out of 150 patients 60 females showed bacterial growth whereas 60 females showedbacterial growth.

Another Study consisting of patients visiting tertiary hospital in Lahore conducted by Bajpai *et al.*, (2017)found out of 259 samples, 96 Males showed positive growth whereas 45 females showed positivebacterial growth.

Out of total 250 specimens processed; 50 specimens met the inclusion criteria. The resistant pattern of *P. auregunisa* revealed Piperacillin as the least effective drug followed by Amoxcillin (Table 3), whereas the most effective antibiotics were Gentamycin and Cotrimoxazole. For *K. Pnuemoniae* Gentamycin and Ciprofloxacin were most effective (Table 4). Gentamycin was considered most effective antibiotics for *E. coli* (Table 5). Cotrimoxazole and Ciprofloxacin were effective for *K. oxytoca* (Table 6).

According to study of Duan *et al* (2020), *Pseudomonas aeruginosa* of LRTI patients remained highly susceptible (>70%) to amikacin, tobramycin, gentamycin and its antibiotic sensitivity to meropenem and imipenem was moderate moderately susceptible to gentamycin in both the adult respiratory ward and RICU and to clindamycin, oxacillin, moxifloxacin only in the adult respiratory ward. Another study found the highest sensitivity to Amikacin (82.14%) followed by Carbapenems (78.57%) (Samad *et al.* (2017). All MDR isolates were resistant to Cefoperazone + Sulbactam. Resistance to Piperacillin + Tazobactam was 96.43%.

Table 2: Gender-wise Distribution of Bacterial Sample

	Significant Growth		**Non-Significant Growth**		**Total**
	Number	**Percentage**	**Number**	**Percentage**	
Male	27	54%	113	56.5%	140
Female	23	46%	87	43.5%	110
Total	50		200		250

Table 3: Antibiotic Susceptibility test for *P. aeruginosa*

Name of Antibiotics	**Sensitive**		**Resistant**	
	Number	**Percentage**	**Number**	**Percentage**
Ciprofloxacin (CIP)	5	29	12	71
Cotrimoxazole (COT)	6	35	11	65
Gentamycin (GEN)	8	47	9	53
Piperacillin (PI)	0	0	17	100
Piperacillin/Tazobactum (PIT)	6	35	11	65
Ceftazidime (CAZ)	2	12	17	88

Table 4: AST pattern for *K. Pneumoniae*

Name of Antibiotics	**Sensitive**		**Resistant**	
	Number	**Percentage**	**Number**	**Percentage**
Amoxicillin (AMX)	6	29	15	71
Cotrimoxazole (COT)	10	48	11	52
Ciprofloxacin (CIP)	17	81	4	19
Gentamycin (GEN)	20	95	1	4
Cefixime (CFM)	13	62	8	38
Cefotaxime (CTX)	17	81	4	19

Table 5: AST pattern for *E. coli*

Name of Antibiotics	**Sensitive**		**Resistant**	
	Number	**Percentage**	**Number**	**Percentage**
Amoxicillin (AMX)	0	0	5	100
Cotrimoxazole (COT)	2	40	3	60
Ciprofloxacin (CIP)	1	20	4	80
Gentamycin (GEN)	4	80	1	20
Cefixime (CFM)	2	40	3	60
Cefotaxime (CTX)	2	40	3	60

Table 6: AST pattern for *K. Oxytoca*

Name of Antibiotics	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
Amoxicillin (AMX)	2	50	2	50
Cotrimoxazole (COT)	3	75	1	25
Ciprofloxacin (CIP)	3	75	1	25
Gentamycin (GEN)	2	50	2	50
Cefixime (CFM)	2	50	2	50
Cefotaxime (CTX)	3	75	1	25

Table 7: MDR and Non-MDR Gram Negative Bacteria

Bacterial Isolates	MDR		Non- MDR	
	Number	Percentage	Number	Percentage
K. pnuemoniae	5	24	16	76
P. aeruginosa	13	76	4	24
E. Coli	3	60	2	40
K. Oxytoca	2	50	2	50

MDR and Non-MDR Bacteria

The data shown in the Table 7 that -among the 50 positive cases, 76 % *P. aeruginosa* were identified as Multi- Drug Resistant (MDR) bacterial isolates followed by 60% of *E. Coli*.

Out of 50 positive growths seen, 27 bacteria show a MDR whereas other were non-MDR which is 87.10% of total sample tested. A study in Nepal found that the ratio of MDR and non-MDR bacterial isolates among positive detected cases of pneumonia was 22.2% to 77.8% according to Shrestha *et al*; (2022), this means that about one in four patients with pneumonia had an MDR bacterial infection. Another study in India found that the ratio of MDR and non-MDR bacterial isolates among positive detected cases of tuberculosis was 33.3% to 66.7%. This study was done by Kumar *et al*; (2021), this means that about one in three patients with tuberculosis had an MDR bacterial infection.

In our study, the high prevalence of MDR bacterial isolates among the positive cases (87.10%) isa concerning finding. This underscores the importance of judicious antibiotic use and the development of novel therapeutic strategies to combat the rising threat of antibiotic resistance in respiratory infections.

MBL and non-MBL producing Gram Negative Bacterial Isolates

The results indicate that 53% MBL producing *Pseudomonas aeruginosa,100% of E. coli, 25%* of *K. oxytoca* and 14% of *K. pneumonia* were detected that strains exhibit reduced susceptibility to imipenem, characterized by smaller zones of inhibition, which can be partially restored when imipenem is combined with EDTA, suggesting the presence of Metallo-Beta-Lactamase (MBL) (Table 8).

According to Kali *et al;* Metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa* has emerged as a threat to hospital infection control, due to its multi-drug resistance, especially in intensive care units (ICUs). Similar result was found by study carried out by Acharya *et al;*(2019), to determine the prevalence of metallo-β-lactamases (MBLs) producing *Pseudomonas aeruginosa* in imipenem-nonsusceptible isolates and to detect MBL-encoding genes among MBLs-positive isolates. Metallo-β-lactamases production was detected in 68.6% isolates of *P. aeruginosa* with reduced susceptibility to imipenem.

From 50 Isolated Bacteria, 28 were ESBL producing bacteria where *E. coli* and *K. oxytoca* shows100% growth followed by *K. pneumonia* (Table 9).

In the study conducted by Horie *et al*; in community acquired pneumonia or Healthcare associated pneumonia, out of 400 patients 27 ESBL producing Bacteria were found.

Another study from Gram Negative Pathogens in Nepal Public Health Laboratory of Nepal conducted for 6 months by Shrestha *et al.* (2022) found a total 47 ESBL producing Bacteria out of total 4226 Clinical Specimens.

Table 8: MBL and non-MBL of Bacterial Isolates

Bacterial Isolates	MBL producers		Non- MBL	
	Number	Percentage	Number	Percentage
K. Pnuemoniae	3	14	18	86
P. aeruginosa	9	53	8	47
E. Coli	5	100	0	-
K. Oxytoca	1	25	3	75

Table 9: ESBL Detection

Bacterial Isolates	ESBL producers		Non- ESBL	
	Number	Percentage	Number	Percentage
K. pnuemoniae	16	76	5	24
P. aeruginosa	3	18	14	82
E. Coli	5	100	0	-
K. Oxytoca	4	100	0	-

Microorganisms Detected

Out of 50 significant growths, 21 were *K. pnuemonae*, 17 were *P. aeruginosa* and *E. Coli* were 5. Fig. 2 shows the findings of the microorganisms:

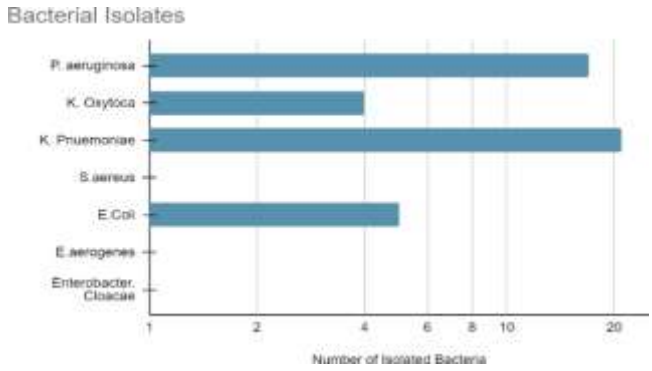

Fig. 2: Bacterial Isolates from significant growth

According to Ibrahim *et al*; the most common pathogens detected with a sputum culture are bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, and *Klebsiella spp*. The analysis from the Department of Microbiologist of KIST Medical College also Suggest *Klebsiella* is most common isolate followed by *Pseudomonas*, *Escherichia coli*, *Acinetobacter*, *Staphylococcus aureus*, *Candida albicans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and others.

Fig. 3: Growth in different culture plate

As sputum specimens are observed for mucopurulent strands, leukocytes, and blood and culture results. The detected organisms can infect the respiratory system and can cause serious infections

A total of 250 sputum specimens were collected from patients at a hospital. Of these, 50 (20%) tested positive for bacterial infection. The most common bacterial isolate was *Pseudomonas aeruginosa, Klebsiella pneumoniae, E. coli*, and *K. oxytoca*.

The age distribution illustrated intriguing patterns, with the age group over 71-80 having the highest representation, followed closely by the 81-90 and 51-60 age group. The gender distribution was done with 54% males and 46% females, potentially indicating gender-related susceptibilities to respiratory infections.

In this comprehensive investigation of sputum samples, we unveiled significant insights into the antibiogram pattern of bacterial infections. Among the key findings, Gentamycin and Cotrimoxazole are most effective to bacterial isolates as *K. Pneumonia*, *P. aeruginosa*, *E. coli* and *K. Oxytoca*. Among the significant growth a significant 87.10% 76 % *P. aeruginosa* were identified as Multi-Drug Resistant (MDR) bacterial isolates followed by 60% of *E. coli*.

The antibiogram pattern observed recently highlights the alarming rise of antibiotic resistance among the clinical isolates tested. The high resistance rates to commonly used antibiotics, such as ampicillin and ciprofloxacin, are particularly concerning as they are often first-line treatments forbacterial infections. This resistance trend is indicative of the overuse and misuse of these antibiotics in both clinical and agricultural settings. The detection of multi-drug resistant (MDR) strains, especially those resistant to carbapenems, underscores the urgent need for stringent antibiotic stewardship programs. These MDR strains pose a significant threat to public health as they limit therapeutic options and increase the risk of treatment

failure. The presence of resistance genes, as confirmed by molecular analysis, further supports the necessity for ongoing surveillanceand the development of new antimicrobial agents. Our findings are consistent with global report of rising antibiotic resistance, emphasizing the critical need for coordinated efforts to curb this growing threat. These results also call for revisiting antibiotic prescribing practices and enhancing infection control measures to mitigate the spread of resistant pathogens.

Author's Contribution

K. Nyaupane & B. Bhainatwo designed the research plan; K. Nyaupane & N. Mahat performed experimental works & collected the required data. All authors jointly analysed the data; K. Nyaupane, N. Mahat, & A. Lamichhane prepared the manuscript. K. Nyaupane & P. Parajuli critically revised and finalized the manuscript. Final form of manuscript was approved by all authors.

Conflict of Interest

The authors declare that they have no conflicts of interest with the present publication.

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